ISSCR

INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

POSTER ABSTRACTS

2013 11TH ANNUAL MEETING
June 12-15, BOSTON, MA USA

Co-Sponsored by

HSCI
HARVARD STEM CELL INSTITUTE

Cellular Dynamics International

Experience the New iCell® Operating System

Human Biology: The iCell O/S is a More Predictive Model

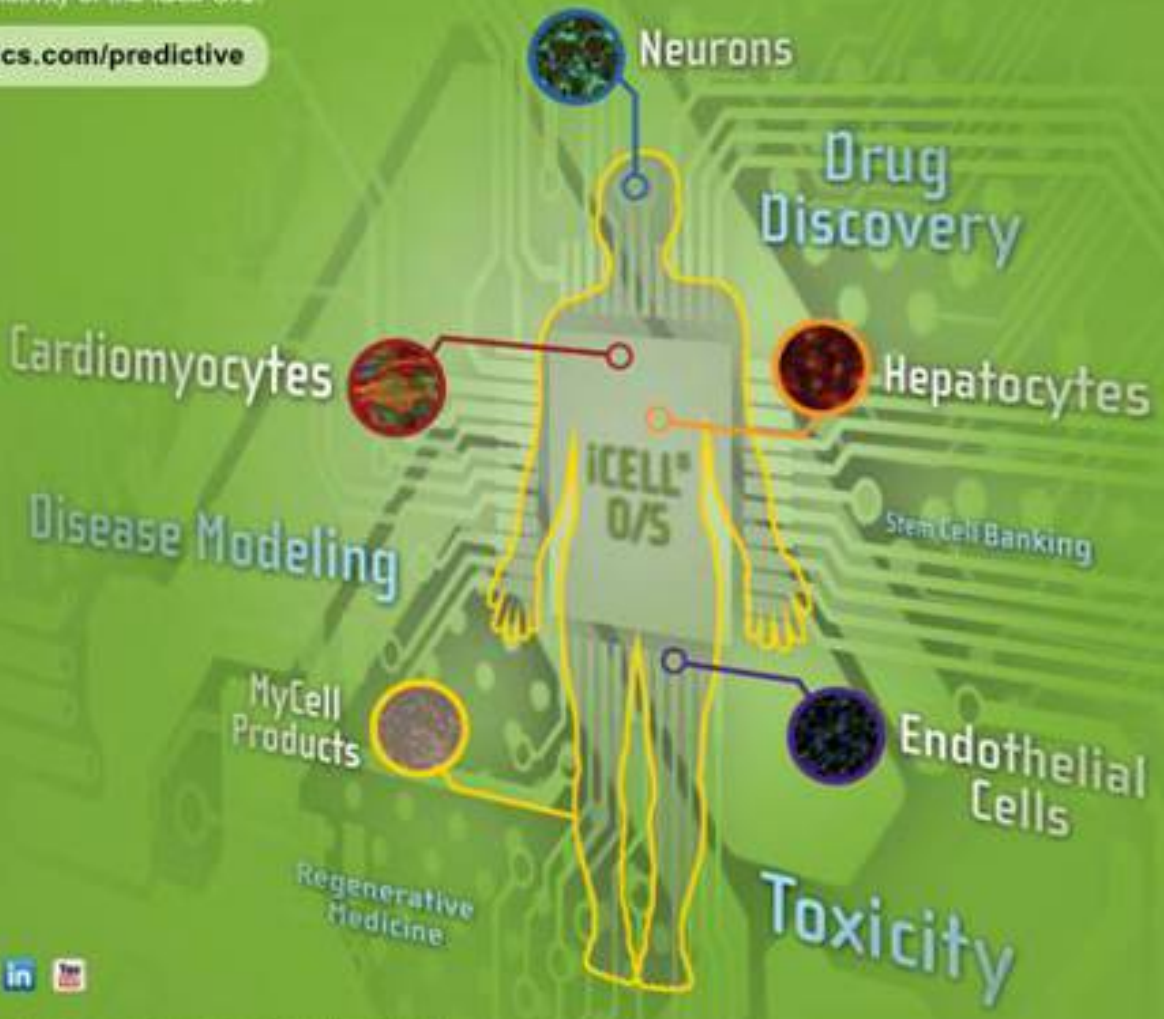
Cellular Dynamics' iPS cell technology enables access to native human biology to power applications in Drug Discovery, Toxicity Testing, Disease Modeling, Stem Cell Banking, and Regenerative Medicine. Select our ready-made cells or submit a donor sample for disease modeling.

Experience the iCell human O/S:

- **Extensively characterized**
- **Consistent & reproducible:** >90% purity
- **Convenient:** Cryopreserved cells + media
- **Proven & published:** Most peer-reviewed citations of any commercial provider

To learn about the predictivity of the iCell O/S:

www.cellulardynamics.com/predictive



Wednesday Poster Abstracts

Education & Outreach; Ethics and Public Policy; Society Issues

Cancer Cells

Chromatin in Stem Cells

Pancreatic Cells

Liver Cells

Intestinal/Gut Cells

Epidermal Cells

Epithelial Cells (not Skin)

Embryonic Stem Cell Clinical Application

Hematopoietic Cells

Endothelial Cells /Hemangioblasts

Other

Technologies for Stem Cell Research

iPS Cells

Reprogramming

Regeneration Mechanisms

Tissue Engineering

Embryonic Stem Cell Pluripotency

Embryonic Stem Cell Differentiation

Totipotent/Early Embryo Cells

Germline Cells

Pre- and Clinical Applications of Mesenchymal Cells

Mesenchymal Cell Lineage Analysis

Mesenchymal Stem Cell Differentiation

Muscle Cells

Cardiac Cells

Eye or Retinal Cells

Neural Cells

Thursday Poster Abstracts

Friday Poster Abstracts

Ethics and Public Policy

Cancer Cells

Chromatin in Stem Cells

Pancreatic Cells

Liver Cells

Intestinal/Gut Cells

Epidermal Cells

Epithelial Cells (not Skin)

Embryonic Stem Cell Clinical Application

Hematopoietic Cells

Society Issues

Endothelial Cells/ Hemangioblasts

Other

Technologies for Stem Cell Research

iPS Cells

Reprogramming

Regeneration Mechanisms

Tissue Engineering

Embryonic Stem Cell Pluripotency

Embryonic Stem Cell Differentiation

Totipotent/Early Embryo Cells

Germline Cells

Pre- and Clinical Applications of Mesenchymal Cells

Mesenchymal Cell Lineage Analysis

Mesenchymal Stem Cell Differentiation

Muscle Cells

Cardiac Cells

Eye or Retinal Cells

Neural Cells

Education and Outreach, Ethics and Public Policy, History of Stem Cell Research, Society Issues

W-1001

#STEMCELLRESEARCH: THE ETHICAL CHALLENGES AND POLICY-MAKING OPPORTUNITIES PRESENTED BY THE USE OF SOCIAL MEDIA IN STEM CELL RESEARCH

Beth E. Roxland[1], Paul Knoepfler[2]

[1]Center for Bioethics at Hofstra University, New York, NY, United States, [2]Univ of California, Davis Sch of Medicine, Sacramento, CA, United States

Since the original derivation of human embryonic stem cells in 1998, much publicity has surrounded the field of stem cell research. In recent years, social media and Internet fora, such as Twitter, Facebook, blogs and patient advocacy and empowerment websites, have played increasingly powerful roles in shaping public perceptions of stem cell research. The unfettered use of the Internet can play both positive and negative roles, however.

Accurate reporting of important discoveries and advances can influence the public's willingness to support stem cell research, to participate in stem cell clinical trials or to be treated with stem cell-based therapies. Effective use of social media may also influence political leaders and legislative efforts, affecting the amount of funding for government-sponsored research as well as the stringency of governmental oversight of research and translational therapies. Professional societies, clinicians and individual researchers also can employ social media as part of their outreach and educational initiatives, which will likely increase in prominence as mainstream media outlets cut funding for dedicated science reporting.

The impact of social media in the stem cell field also may have negative effects. One example is the emerging trend of for-profit stem cell clinics and doctors recruiting patients on the Internet through social media - a forum that often eludes regulators and watchdog groups - by using unsubstantiated claims of efficacy and safety of stem cell products. Similarly, social media may be used to promote stem cell tourism - where patients will travel to other jurisdictions (foreign, or even domestic) that have more lenient laws and oversight of stem cell therapies - for interventions that may be ineffective at best, harmful at worst. Finally, patients (or, people posing as patients) also use social media to advocate in favor of specific for-profit clinics and challenge regulatory oversight.

People suffering from illness, who may be in a particularly vulnerable situation, often turn to the internet to educate themselves about their medical condition and available treatment therapies. Relying on social media rather than trained, unbiased, and regulated sources for information is a dangerous proposition, however. In addition to intentional deception by those who may financially profit from certain research or therapeutic endeavors, some well-meaning people who attempt to educate others via social media on an ad hoc basis may unintentionally spread misconceptions about stem cells. Further, patients often attempt directly to engage stem cell researchers or clinicians through social media, leaving the trained experts uncertain of how - or even whether - to respond.

The rapidly-evolving use of social media in the stem cell field presents ethical and legal issues that the field cannot afford to ignore. In this presentation, we discuss the moral and policy challenges presented by the use of social media as stem cell research moves from bench to bedside. We also discuss ways in which governmental entities, stem cell researchers and clinicians, and professional societies may use social media as a tool to enhance public education of the state of stem cell research, and temper the hype that has surrounded this research for well over a decade. Finally, we suggest a framework to guide the use of social media by those in the field of stem cell research.

W-1002

ETHICAL CONTROVERSY, GRADUATE EDUCATION AND THE DEVELOPMENT OF SCIENTIFIC CAREERS

Ruchir N. Karmali[1], Matthew D. Pjecha[1], **Aaron D. Levine**[2]

[A]School of Public Policy, [B]School of Public Policy, Institute of Bioengineering & Bioscience, [1]Georgia Tech, Atlanta, GA, United States

Scientific advances in diverse fields ranging from stem cell research to nanotechnology pose challenging ethical questions. At the same time efforts to strengthen and broaden the U.S. workforce in science, technology, engineering and mathematics are crucial to the long-term competitiveness of the national economy. This research examines the nexus of these two trends, assessing the extent to which and the manner by which ethical controversy affects the training of graduate students and their

transition to the scientific workforce. Insights into both the challenges and the benefits of training in an ethically contentious field are gathered through interviews with early career scientists in both stem cell science and bio-nanotechology. Participants in these wide-ranging interviews were asked to reflect on the ethics of their own research, their sense of the public perceptions of their research and the various impacts of working in areas that raised ethical concerns, for at least some people, on their research. Preliminary thematic analysis of interview transcripts revealed mismatches between scientists' perceptions of the ethics of their work and their beliefs about public perceptions. In particular, in the context of stem cell science, scientists expressed worries about animal research or human biopsies (e.g. for induced pluripotent stem cell derivation) at about the same rate as they expressed concerns about the use of embryos in research, while discussion of public perceptions of the field focused on concerns about human embryos. Scientists in contentious fields reported a range of funding challenges, some of which they attributed to ethical controversy and the resulting policy environment surrounding their field, rather than broader funding trends. Scientists also reported changing research topics or avoiding certain topics altogether due to policy concerns and frequently noted that scientists working in ethically contentious fields must navigate a complex bureaucracy to complete their work. Scientists also noted what they perceived as greater need to engage in public outreach in ethically contentious fields. The research concludes by drawing on this improved understanding of the experience of scientists in fields that raise ethical controversy to suggest strategies to strengthen graduate education in stem cell science and similar fields.

W-1003

WHAT IS THE ROLE OF REDUCED FEES IN PERSUADING IVF PATIENTS TO VOLUNTEER TO PROVIDE EGGS FOR SCNT RESEARCH?

Erica Haimes
Policy, Ethics and Life Sciences Research Centre, Newcastle University, Newcastle Upon Tyne, United Kingdom

This paper reports selected findings from a UK study investigating the question: 'Does volunteering for the 'Newcastle egg sharing for research scheme', in which IVF patients pay reduced fees when providing 50% of their fresh eggs to research, entail any social and ethical costs?'. This study fills a gap in knowledge, as there have been no previous systematic investigations of women's experiences of volunteering to provide eggs for research under such circumstances. The focus in this presentation is on women's views of the role of the reduced fees in persuading them to volunteer in the first place. This was an interview-based, interpretivist study, designed to gain understanding of the volunteers' reasoning and experiences. The main findings indicate that the interviewees' goal is to have a baby; they volunteered to provide eggs to research to gain access to cheaper treatment, in a context where private IVF fees are high and there is insufficient state funding. However, the decision to volunteer was not easy, since interviewees had to juggle a wide range of uncertainties about the consequent gains and losses, when already experiencing the uncertain world of the 'IVF rollercoaster'. Overall, interviewees welcomed the 'egg sharing' scheme, but were not volunteering entirely under circumstances of their own choosing. For example, they would prefer not to provide fresh eggs whilst going through treatment and decided not to do so, under certain circumstances. They also resisted the opportunity to provide eggs for the treatment of other couples, even though they would have paid even lower fees by so doing. Therefore, although important, reduced fees do not act as an undue inducement, persuading volunteers to act against their own interests.

W-1004

COLLABORATION IN STEM CELL RESEARCH: A PHILOSOPHICAL ACCOUNT

Melinda B. Fagan
Philosophy, Rice Univ, Houston, TX, United States

I present results of a philosophical study of stem cell research. The goal of the study was to articulate conceptual issues related to evidence, experiment, and explanation. Methods used are those of philosophy: conceptual analysis, reflection on past and contemporary cases, and logical argument. The full argument for a philosophical audience appears in a new book: Philosophy of Stem Cell Biology, Palgrave Macmillan (Jan 2013). The present study differs from the book in focus and presentation, extending the results for an audience of stem cell scientists. Here, my overall claim is that collaboration among stem cell researchers is more fundamental, and more complex, than has been explicitly recognized. I support this claim with specific case studies and arguments.
First, collaboration among laboratories using different methods is necessary to support any general claims about stem cells. This follows from the general definition of a stem cell as a cell that is capable of both self-renewal and differentiation. When precisely expressed as an abstract model, this stem cell concept is shown to depend on several parameters, differently specified by different experimental methods: a cell lineage L, time interval n, and set of variable cell characters C. To apply to biological phenomena, the key parameters must be specified. Different values of these variables correspond to different entities that can be identified as 'stem cells.' This accounts for the diversity of entities referred to as 'stem cells.' Another consequence is that claims about stem cells must be understood in terms of experimental methods used to identify them. The question then arises: how do these disparate results fit together to yield a satisfying explanation? Explanatory generalizations about stem cells, and clinical applications based thereon, require collaboration across experimental contexts.
This collaborative view of stem cell research has several important implications. One is that the distinction between 'adult' and 'embryonic' stem cell research is revealed as pernicious. Case studies of mHSC, hESC, and teratocarcinoma/EC research indicate that field as a whole progresses by multiplying models and constructing a network of comparisons among them. Though hESC research plays a pivotal role in this research network, general knowledge emerges from 'traffic' across models and new combinations of techniques. The next step forward is to unify the two main branches of stem cell biology, expanding the network of comparisons and generality of results. The 'adult/embryonic' dualism impedes this progress. Another consequence of my view is that Systems Biology (specifically, mathematical models of gene regulatory networks) has a key role to play in explanations of stem cell phenomena, as do simple abstract models like Waddington's epigenetic landscape. Thirdly, the network of stem cell models and methods is structured and unified by clinical goals, which thereby influence emerging explanations. This unifying, guiding role is analogous to fundamental theory in physics. This suggests a general standard for responsible translational research, that would be accessible to non-experts. So these philosophical arguments, though abstract, have important implications for organization of stem cell research and progress toward clinical translation.

W-1005

POLICY UNCERTAINTY, RESEARCH FUNDING AND THE CONDUCT OF STEM CELL SCIENCE

Aaron D. Levine
School of Public Policy, Institute of Bioengineering & Bioscience, Georgia Tech, Atlanta, GA, United States

Emerging technologies, such as stem cell research and nanotechnology, offer substantial promise to improve our quality of life. Yet, at the same time, many emerging technologies raise challenging ethical questions. As a result of these and other tensions, the outcomes of policy debates surrounding emerging technologies often vary both across jurisdictions and time, yielding a heterogeneous and uncertain policy environment. In this project, I consider policy uncertainty surrounding pluripotent stem cell research and assess its impact on scientists in this promising but controversial field. Drawing on data from a recent survey of stem cell scientists in the United States, I argue that policy uncertainty surrounding federal funding policies for human embryonic stem cell research has negatively impacted the development of the broader field of stem cell science, affecting not just scientists working with human embryonic stem cells, but also those working with less controversial types of stem cells. My analysis also illustrates the importance of state policy in this field. In particular, scientists in states that have provided funding for stem

cell research, including research using human embryonic stem cells, are substantially less likely than scientists in states without such funding to report impacts of policy uncertainty. This effect is present for the full sample of stem cell scientists, including both scientists working with human embryonic stem cells and other less contentious cell types, but is strongest for scientists working with human embryonic stem cells. State policies that legalize or otherwise support stem cell research, but do not provide funding have no discernible effect. Together results highlight the negative consequences of the policy uncertainly on the development of stem cell research and emphasize the importance of developing and maintaining stable funding structures in ethically contentious areas of biomedical research.

W-1007

SELF REGULATION OF AUTOLOGOUS CELL THERAPIES: A MODEL

Bernard E. Tuch, Heather Main, Dominic Wall
NSW Stem Cell Network, Sydney, Australia

Autologous cell therapies are being practised by an increasing number of clinicians internationally - for the treatment of a variety of disorders. This includes rheumatological conditions where the sources of cells are the vascular stromal fraction obtained from lipoaspirates during liposuction, and platelet rich plasma obtained from peripheral blood. In Australia, oversight of these procedures by the regulatory body, the Therapeutic Goods Administration (TGA), is not required. This is because the current regulations contains a broad exemption permitting clinicians to treat their patients with autologous cells without restrictions on the extent of manipulation or whether the cells are used in a homologous manner.

Despite this lack of oversight, a number of companies carrying out autologous cell therapies have become concerned about the lack of checks and balances. End users also have their welfare to be considered. To address these concerns, the NSW Stem Cell Network held a Workshop entitled Cellular Therapies for Repair of Musculoskeletal Injuries in Sydney during October 2012. Four key companies practising autologous therapies for rheumatological conditions presented their data, as did Mesoblast, which is conducting clinical trials using allogeneic cells. A representative of the TGA spoke about the regulatory requirements, and appropriate cell manufacturing requirements were addressed by a representative of the International Society for Cellular Therapy (ISCT).

Out of the workshop came a desire for the establishment of a Steering Committee to discuss self regulation by the companies practising autologous therapies. Three teleconferences have occurred between then and the date this abstract was written in February 2013, chaired by the Director of the NSW Stem Cell Network and involving the ISCT local representative. Issues raised and agreed to have been (a) the need for the development of evidence based medicine; (b) ensuring fully informed consent is obtained; (c) manufacturing the autologous product to be injected into the patient using internationally accepted standards, such as those produced by the Foundation for the Accreditation of Cellular Therapy; (d) following the advertising standards set by the Australian Health Practitioner Regulation Agency.

To cement these agreed-to standards into practice, it has now been decided by the members of the Steering Committee to create a written Code of Conduct. It is anticipated that this Code of Conduct will include the mechanisms of implementing the necessary checks and balances to provide guidance to its users, as well as independently monitoring the practices being followed. The input of consumers also will be needed.

Whilst there is much work to be done in the creation and implementation of the Code of Conduct, it represents a step in the necessary direction to ensure high standards of health care are maintained in a field with a low level of government regulation. Self regulation is possible in this industry, just as it is practised in many others, for example, Medicines Australia to some extent regulates how pharmaceutical companies operate in our country.

Cancer Cells

W-1011

ADAM10 AND ADAM17 INCREASE MIGRATION OF GLIOBLASTOMA STEM PROGENITOR CELLS

Elodie J. Siney, Gareth J. Thomas, **Sandrine Willaime-Morawek**
Faculty of Medicine, University of Southampton, Southampton, United Kingdom

Glioblastoma (GBM) is the most aggressive form of malignant brain tumour, with a mean survival of just over one year. High recurrence rates after surgical resection and ineffectiveness of chemotherapy have suggested a role for brain tumour stem cells (BTSCs) in producing resistance to therapy. To improve this poor prognosis there is a need to develop new adjuvant therapies to slow disease progression, specifically reducing the invasion of neoplastic cells into healthy brain tissue. Due to their role in tissue remodelling, members of *A D*isintegrin *A*nd *M*etalloproteinase (ADAM) family have been implicated in the etiology of solid tumours including breast, colon and glioma, where reports of overexpression of ADAM10 and 17 have prompted further investigation into the role of the proteins in glioma biology, tumourigenicity and invasion. We previously showed that ADAM10 and 17 are overexpressed in glioblastoma and promote BTSCs stemness properties by supporting their self-renewal. We now examine the effect of ADAM inhibition on GBM stem/progenitor cell migration and differentiation *in vitro*. Here we report that inhibition of ADAM10 and 17 increases glioma cell migration towards a mix of growth factors, including epidermal growth factor and basic fibroblastic growth factor. Migrated BTSCs exhibited decreased stem cell/progenitor markers (Nestin and CD133), but increased neuronal lineage marker (beta-III-tubulin) expression compared to non-migrated cells suggesting that migrated cells are more differentiated.
Using a 3D *in vitro* model, we also showed that ADAM10 and 17 inhibition promotes migration of cells out of the tumourspheres. Surprisingly, in this model, the migrated cells seems less differentiated, showing an increase of nestin expression and a decrease of beta-III-tubulin expression, compared to cells closer to the centre of the spheres. These results suggest a role for ADAMs in maintaining a BTSC phenotype and give insight into possible therapies that target the invading GBM cells.

W-1012

SIGNALS OF CD44/HYALURONAN PROMOTES HEPATOCELLULAR CARCINOMA STEM CELL PROLIFERATION AND PRODUCTION OF ALPHA FETOPROTEIN IN VITRO

Hsiao-Nan Hao[1], Qingbao Wang[2], Jean Peduzzi[1], Peter Hao[1], Deqi Xiang[3]
[1]Wayne State University, Detroit, MI, United States, [2]surgery, Taian medical college, Taian, Shandong, China, [3]Xiamen Neocell Biotech, Xieman, China

Introduction: Cancer stem cells (CSC) have recently been proposed to be cancer initiating cells responsible for tumorigenesis and contributing to cancer resistance. CSCs have been considered to be a cardinal cellular target for cancer chemotherapy because a single CSC plays a critical role in generating malignant tumors under certain circumstances. CSCs share many of cell biological characteristics with non-cancer stem cells such as self-renewal and proliferation as well as differentiation. Hepatocellular carcinoma is a solid common malignant tumor arising from the hepatocytes in liver tissue. Many reported results have shown that hepatoblastoma stained heterogeneously. However, among the cancer tissue cell isolates, a small population of cells interacts positively with stem cell makers such as CD34 and CD44. Those cells were also strongly stained with a-fetoprotein. The CD44/hyaluronan molecules on the surface of CSC have been found to play important roles on tumor initiation, proliferation, metastasis as well as tumor invasion induced inflammation. In this study, we have characterized the expression of the CD44/hyaluronan on the isolate liver cancer cells. Enriched CD44/hyaluronan expressed on and in the surface membrane and cytosol specifically in vitro. The results of this study also demonstrate that the a-fetoprotein production of CSC highly associate with CD44 expression. Lower levels of CD44/hyaluronan is unlikely to up-regulate the CSC to express a-fetoprotein, whereas, high levels of CD44/hyaluronan affects a-fetoprotein strongly. Data from in vitro studies have shown that enhancement of CD44 on the surface occurs only in the cells with CD34/CD44 dual expression.

This experimental result provides a new methodology to identify and isolate CSC from hepatoblastoma surgical specimens. However, the mechanism of CD44 to regulate alpha fetoprotein expression in the population of CSC-like cells needs further studies.
Experimental Results: This study focused on a methodology to identify and isolate CSC from of hepatoblastoma surgical specimens. Viable cells of hepatoblastoma were incubated with specified MEM culture medium. CD34, CD44, hyaluronan, keratin AE1/AE3, CAM5, CK7, CEA and alpha fetoprotein were analyzed using immunocytochemical and laser scanning microscope. The percentage of CSC was estimated. Overall, about 5% to 8% cells (from 22 clinical specimens) were defined to be CSC according to the stem cell specific makers. The gene expression profile of CSC was also analyzed use cDNA microarray representing 11000 mRNAs. Membrane and cytosolic protein were analyzed use western blotting and flow cytometer. Endosomal CD44 and hyaluronan were also estimated. The results indicate that non-CSC can cells show a lack of evidence to have CD34/CD44/hyaluronan involved although it is likely to produce detectable amount alpha fetoprotein. The in vitro results have shown that increasing alpha fetoprotein expression significantly follows elevated the levels of CD44/ hyaluronan.
Material Methods: This is an on-going CSC research project that was authorized by university HIC. Cancer cells were isolated from surgical specimens after pathology diagnosis. The human fetal liver cell primary cultures were used for alpha fetoprotein production control. The viability of all cell isolates is more than 90% used for this study. CSC mRNA profiling was analyzed use micro-array. The intracellular levels of alpha fetoprotein and CEA were estimated use ELISA.

W-1013

THE MET ONCOGENE IS A FUNCTIONAL MARKER OF A GLIOBLASTOMA STEM CELL SUBTYPE AND A THERAPEUTICAL TARGET FOR RADIOSENSITIZATION

Carla Boccaccio[1], Francesca De Bacco[1], Elena Casanova[1], Enzo Medico[1], Serena Pellegatta[2], Francesca Orzan[1], Raffaella Albano[1], Paolo Luraghi[1], Antonio D'Ambrosio[1], Bianca Pollo[2], Paolo M. Comoglio[1], Gaetano Finocchiaro[2]
[1]Institute for Cancer Research, Candiolo, Italy, [2]Fondazione IRCCS-Istituto Neurologico C. Besta, Milano, Italy

The existence of treatment-resistant cancer stem cells contributes to the aggressive phenotype of glioblastoma. However, the molecular mechanisms that sustain stem cell proliferation in these tumors are largely unknown. We report that expression of the MET oncogene, encoding Hepatocyte Growth Factor (HGF) Receptor, identifies a subset of glioblastoma-derived neurospheres (MET-pos-NS), characterized by distinctive genetic and molecular features such as: (i) normal EGF Receptor gene copy number; (ii) inactivation of the tumour suppressor gene PTEN; (iii) gene expression profile classified as “mesenchymal” or “proneural”. Conversely, neurospheres that do not express MET (MET-neg-NS) usually display complementary features such as: (i) EGF Receptor gene amplification; (ii) intact PTEN gene; (iii) gene expression profile defined as “classical”. We show that MET-pos-NS are endowed with a hierarchical organization, including a cell subpopulation expressing high levels of MET (MET-high) at the top, and a cell subpopulation that conversely express negligible levels of MET (MET-low), at the base. This conclusion is supported by data showing that, upon isolation from neurospheres, (i) MET-high, but not MET-low cells, retain long-term propagation and multi-potential differentiation; (ii) MET-high, but not MET-low cells, can regenerate a mixed MET-high/MET-low cell population; (iii) MET-high display increased tumorigenic ability as compared to MET-low cells, and are the only that form tumors containing both MET-high and MET-low cells. In MET-high cells, we find also that HGF sustains proliferation, clonogenicity, expression of self-renewal markers, migration, and invasion in vitro. Finally, we show that MET-high cells are more radioresistant than MET-neg cells, and are dramatically radiosensitized by treatment with specific MET inhibitors. Together, these findings suggest that MET is a functional marker of glioblastoma stem cells, and a promising target for identification and therapy of a subset of glioblastomas.

W-1014

MESENCHYMAL TRANSFORMATION OF GLIOMA STEM CELLS IS REGULATED BY THE ALDEHYDE DEHYDROGENASE 1A3-MIR99B/LET7E/125A SIGNALING IN A C/EBPB-DEPENDENT MANNER

Sunghak Kim[1], Ping Mao[1], Kaushal Joshi[1], Jianfeng Li[2], Lucas Santana-Santos[3], Uma R. Chandran[3], Panayiotis Benos[3], Luke Smith[1], Mao Dewang[4], Bo Hu[5], Shi-Yuan Cheng[5], Robert W. Sobol[3], ichiro Nakano[1]

[1]Ohio State University, Columbus, OH, United States, [2]University of Pittsburge, Pittsburgh, PA, United States, [3]University of Pittsburgh, Pittsburgh, PA, United States, [4]Xi'an Jiaotong University, Xi'an, China, [5]Northwestern University, Chicago, IL, United States

High-grade glioma (HGG) tumors are composed of heterogenous tumor cell populations including tumor cells with stem cell properties termed glioma initiating/propagating cells or glioma stem cells (GSCs). Here we present data describing the presence of two distinct GSC subtypes, termed as Proneural (PN) and Mesenchymal (MES) GSCs, in HGGs based upon genome-wide transcriptome microarray analysis and microRNA profiling. Similar to the clinical feature, MES GSCs display more aggressive and radioresistant phenotype than PN GSCs. Intriguingly, PN GSCs acquire MES signature when they are treated with radiation, suggesting the PN-to-MES transition (PMT) of GSCs. Pathway analysis of the array data identified aldehyde dehydrogenase 1A3 (ALDH1A3)-mediated pathway as the most upregulated pathway in MES GSCs over PN GSCs. Mechanistically, ALDH1A3 is regulated by the oncogenic transcription factor C/EBPβ by both directly through the binding to the ALDH1A3 promoter and indirectly through regulation of the evolutionary conserved miR cluster containing miR-99b, let-7e, and miR-125a. Physiologically, forced expression of miR-99b-let7e-125a cluster in the MES GSCs leads to the loss of mesenchymal phenotypes and sensitizes MES GSCs to irradiation. Collectively, the C/EBPβ-miR cluster-ALDH1A3 axis plays a critical role in GSC evolution to gain therapy-refractory mesenchymal phenotype.

W-1015

ROLE OF PRION PROTEIN AND STRESS INDUCIBLE PROTEIN ONE INTERACTION IN THE BIOLOGY OF GLIOBLASTOMA STEM CELLS

Rebeca P. Iglesia[1], Tiago G. dos Santos[2], Vilma R. Martins[2], Marilene H. Lopes[1]

[1]Department of Cell Biology and Development, Institute of Biomedical Sciences, São Paulo, Brazil, [2]Laboratory of Cellular and Molecular Biology, A.C. Camargo Hospital, São Paulo, Brazil

Glioblastomas (GBM) are highly lethal brain tumors, which contains a subpopulation of glioblastoma stem cells (GSC) capable of promoting tumor angiogenesis, invasion and radiotherapy and chemotherapy resistance, contributing to the failure to treat these cancers. Studies show that the interaction of two proteins, the cellular prion protein (PrPC) and stress inducible protein 1 (STI1), promote proliferation of GBM and recent data from our group indicate that the blocking of the PrPC-STI1 interaction may have therapeutic potential. Both PrPC and STI1 are highly expressed in the CNS. Their interaction modulates processes involved in neural plasticity and plays an important role in the biology of neural stem cells (NSC), promoting proliferation and self-renewal. Based on these data, this study aims to evaluate the role of PrPC-STI1 interaction in the biology of GSC. Our data suggest that the PrPC-STI1 interaction is involved in GSC self-renewal and proliferation, in which the addition of STI1 in culture medium in clonal density and BrdU incorporation assays seems to promote dose-dependent proliferation in GBM human strain. However, PrPC-nonexpressing cells didn’t show response to treatment with STI1, suggesting that the proliferative effect is dependent on interaction with PrPC. The elucidation of mechanisms controlling GSC proliferation is necessary to identify relevant targets for therapeutic intervention, increasing the effectiveness of treatment through the targeting of GSC, decreasing tumor invasion, proliferation, and even the recurrence of this tumor.

W-1016

EVOLUTION OF CANCER STEM CELLS IN GLIOMA TO PROMOTE THEIR THERAPY RESISTANT PHENOTYPE

Ichiro Nakano

Neurological Surgery, The Ohio State University, Columbus, OH, United States

EVOLUTION OF CANCER STEM CELLS IN GLIOMA TO PROMOTE THEIR THERAPY RESISTANT PHENOTYPE

High grade glioma (HGG) is a life-threatening brain tumor. Recurrence is inevitable in virtually all cases and a permanent cure for HGGs remains elusive. Among heterogeneous tumor cells, glioma stem cells (GSCs) are defined as a subpopulation resistant to chemo- and radiotherapy with prominent tumorigenic ability. Identification of novel therapeutics designed to target the GSC population in HGGs has emerged as an attractive strategy to overcome this lethal disease. The recent genome-wide transcriptome analysis identified 3 subtypes, proneural (PN), proliferative, and mesenchymal (MES), in HGGs. These HGG subtypes harbor distinct gene signatures and altered signaling pathways with some clinical relevance. During malignant transformation, HGGs appear to gain therapy resistance, at least in part, through their phenotypic drift into mesenchymal tumors. In addition, some of the current therapies, if not all, appear to induce mesenchymal transformation of HGGs resulting in more therapy-resistant tumors. Nonetheless, identification of subtype-specific GSCs remains elusive.
My lab recently identified clinical HGG sample-derived two mutually-exclusive GSCs: proneural (PN) and mesenchymal (MES) with striking phenotypic differences with clinical relevance. Transcritpome microarray analysis, miRNA profiling, and subsequent functional analysis demonstrated that distinct signaling pathways regulate tumorigenesis and propagation of the individual GSC subtypes. For example, PN GSCs are dependent on the oncogenic transcription factor c-Myb (Miyazaki et al., Clinical Cancer Res. 2012, and unpublished data) and MES GSCs are dependent on the oncogene/mitotic kinase MELK and Survivin (Guvenc et al., Clinical Cancer Res. 2012; Gu et al., Stem Cells 2013; Joshi et al., Stem Cells 2013). Intriguingly, upon radiation treatment, PN GSCs gain the phenotypes of MES GSCs (PN-to-MES transformation; PMT), which are much more aggressive and radioresistant than original PN GSCs. Of note, targeting the MES-specific pathways partially blocks PMT of GSCs. These results may partly explain the molecular mechanisms underlying mesenchymal transformation of HGGs to promote therapy resistance. Here I summarize our latest data to elucidate the molecular mechanisms that drive GSC evolution to promote their therapy resistant phenotype.

W-1017

THE SEMAPHORIN 3A-NEUROPILIN-1 SIGNALING PROMOTES CLONOGENIC GROWTH OF GLIOMA STEM CELLS VIA ACTIVATION OF TGFβ SIGNALING.

Dong-Hun Woo[1], Nakho Chang[2], Eunhee Kim[1], Jeremy Rich[1], Do-Hyun Nam[2], Jeongwu Lee[1]
[1]Department of Stem Cell Biology and Regenerative Medicine, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, United States, [2]Cancer Stem Cell Research Center, Samsung Biomedical Research Institute, Seoul, Korea, Republic of

Glioblastoma multiforme (GBM) harbors highly tumorigenic, stem-like cells (GBM stem/propagating cells, GSCs) that are implicated in GBM progression and recurrence. Through in vitro clonogenic growth-based screening assays, we have identified Semaphorin 3A (Sema3A) as a potent mitogen for GSCs. Sema3A, generally known as an axon guidance molecule, promotes proliferation and clonogenic growth of GBM cells by binding to its receptor Neuropilin-1 (NRP1). The Sema3A-NRP1 interaction induces phosphorylation of SMAD2 and activates the Transforming growth factor β (TGFβ) signaling, a positive GSC regulator. Freshly isolated patient-derived GBM cells that express high levels of NRP1 proteins are highly clonogenic compared to bulk tumor cells, and enriched with the activated TGFβ signaling pathway. NRP1 inhibition by short hairpin RNA-mediated knockdown or treatment with NRP1 blocking antibodies decreased TGFβ pathway activity and stem cell marker expression in GSCs. Finally, NRP1 knockdown decreased clonogenic growth of GBM cells and prolonged the survival of mice in an orthotopic xenograft tumor model. Together, these data suggest that the Sema3A-NRP1-TGFβ signaling axis is a critical regulator of GSC maintenance and a potential therapeutic target for GBM.

W-1018

S100A4 EXPRESSION AND FUNCTION IN GBM STEM CELLS

Kin-Hoe Chow, Hee Jung Park, Kyuson Yun
Research: Kyuson Yun's lab, The Jackson Laboratory, Bar Harbor, ME, United States

The ineffectiveness of current treatment for the most aggressive form of adult brain tumor, *Glioblastoma multiforme* (GBM), demands novel therapeutic approaches. One such approach may be targeting cancer stem cells (CSCs: a sub-population of cancer cells with defining characteristics of stem cell and the unique ability to initiate a tumor upon transplantation). CSCs have been shown to be more therapy-resistant and have the ability to generate recurring tumors. Unfortunately, the number of known markers and regulators of GBM stem cells is limited currently. Through comparative gene expression analysis of FACS-sorted populations of cells from a mouse GBM model, our laboratory has previously identified 45 genes that show differential expression patterns in GBM stem cells. Here, we focus on analyzing one of these genes, *S100a4*, and provide evidence that *S100a4* is a novel regulator of glioma stem cells. Analysis of mouse GBM tumor tissues confirmed that only a small number of tumor cells express high levels of S100a4, implying that S100a4 could be a candidate marker for CSCs. Using primary tumor cells isolated from S100ß-verbB;p53 gliomas, we found that S100a4^{hi} cells are enriched with self-renewing, sphere-forming cells. In addition, orthotopic injection of S100a4^{hi} and S100a4^{lo} cells from the same tumor showed that mice injected with S100a4^{hi} cells had significantly shorter survival time, supporting the idea that S100a4^{hi} cells are enriched with CSCs. Consistently, when S100a4+ cell were depleted in primary GBM tumorsphere cultures, numbers of self-renewing cells were significantly reduced. Furthermore, when S100a4^{hi} cells were ablated *ex vivo* prior to intracranial injection, mice injected with S100a4^{hi}-depleted cultures survived significantly longer than mice injected with control treated cells. Altogether, these studies strongly support our original hypothesis that S100a4^{hi} cells are enriched with CSCs. Suggestive of conserved function and expression in human GBMs, analysis of a human glioma database (Rembrandt) revealed a significant correlation between *S100A4* expression level and patient survival time. Results from on-going experiments with patient-derived GBM stem cells suggest that *S100A4* is critical for proliferation and self-renewal of human GBM tumorsphere cells *ex vivo*. Collectively, our studies strongly suggest that *S100a4* is a potential regulator and a marker for GBM stem cells in human and mouse tumors.

W-1021

BMP INHIBITORS REGULATE THE DIFFERENTIATION/SELF-RENEWAL AXIS IN GLIOMA STEM CELLS

Kenneth Yan, Anita Hjelmeland, Jeremy Rich
Stem Cell Biology & Regenerative Medicine, Cleveland Clinic, Cleveland, OH, United States

Contributing to the tumorigenicity of glioblastoma are glioma stem cells (GSCs), a population of cells within a given tumor that can propagate heterogeneous tumors and are capable of extensive self-renewal. These cells are known to confer resistance to traditional chemo- and radiotherapies, and so there are hopes that further characterization of these cells will lead to the development of GSC-specific approaches to therapy in the future. Bone morphogenetic proteins (BMPs) are thought to be one potential GSC-specific therapy that will promote GSC differentiation toward an astrocytic lineage and attenuate tumor growth. In designing therapies, however, it is also important to consider secreted microenvironmental cues that may play a major role in tumor growth. These cues are provided within GSC niches by the GSCs themselves or by supporting cells such as tumor-associated microglia or endothelial cells. I have recently identified BMP regulators as potential secreted cues to maintain GSCs. My data demonstrate that these regulators are elevated in the GSC population compared to non-stem glioma cells. I have also found that recombinant forms of these proteins antagonize the effects of exogenous BMP. Furthermore, expression of these proteins correlates with patient survival in certain glioma subtypes. Together, these data suggest that these BMP regulators might be secreted within GSC niches in gliomas, limiting the utility of BMP as a treatment while providing new avenues for targeted therapies.

W-1022

GLIOMA STEM CELL - ENDOTHELIAL CELL NICHE INTERACTIONS DEFINE MALIGNANT PROGRESSION IN GLIOBLASTOMA

Anjali Shiras, Aman Sharma
National Centre for Cell Science, Maharashtra, India

Glioblastoma multiform (GBM) is a highly heterogeneous, vascularized tumor exhibiting hierarchical organization and replete with a distinct glioma stem cell (GSC) population that drives tumor growth, progression and therapeutic resistance. With an aim to identify origin of the self-renewing glioma stem cell population, we developed several *in vitro* model systems that mimic phenomena of malignant tumor progression in GBM. Further, we explored glioma stem cells developed as free floating neurospheres to study mutual interactions with the vascular niche micro-environment that is mainly comprised of endothelial cells (EC) and the perivascular niche components of extracellular matrix. For this, we generated *in vitro* co-culture systems comprising of GSCs and Endothelial cells (EC). We specifically focused on investigating interactions of GSCs with perivascular niche endothelial cells under both two-dimensional and three dimensional growth conditions. Recent reports have shown prominence of ECs in regulation of GSC, however, almost all studies have used either HUVEC or other types of non-tumor derived endothelial cultures. To mimic a more functional vascular niche for GSC and to understand mutual niche specific signalling mechanisms for GSC phenotype maintenance and GSC
driven angiogenesis response in ECs, we independently generated GSC and EC cultures from the same glioma tumor. We differentially labeled GSC and EC cultures with green and red PKH dyes and established GFP/RFP labeled GSC/EC co-culture systems and used them to analyze effects on cell proliferation and angiogenesis using flow cytometry, matrigel tube formation and *in vitro* 3D sprouting angiogenesis assays. GSCs mutually grown with ECs induced enhanced proliferation, migration and contributed towards accelerated tumorigenicity of GSCs, compared to GSC alone. Characteristically, their niche specific mutual interactions induced activation of PDGF signalling and contributed to enhanced proliferation and migration of either cell types in a 3D co-culture assay. Further, we showed that tumor derived endothelial cell population differed significantly from the non-tumor endothelial cells and showed auto-activation of angiogenesis pathways. Interestingly, in PKH labeled co-culture assay, the presence of dual labeled cell population of GSC-EC suggested towards activation of novel niche specific signalling mechanisms mediated by circulating microvesicles**.** We propose that targeting glioma stem cell niche may act as double edge sword and not only curb GSC growth but also inhibit
GSC induced angiogenesis thereby providing novel therapeutic approaches in glioma treatment.

W-1023

DOPAMINE RECEPTOR ANTAGONISTS SELECTIVELY TARGETS THE GROWTH OF GLIOBLASTOMA STEM CELLS.

Sonam Dolma[1], Milly So[2], Ian Clarke[2], Peter Dirks[2]

[1]Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada, [2]Development and Stem Cell, The Hospital for Sick Children, Toronto, ON, Canada

Glioblastoma multiforme (GBM) is the most common malignant primary brain tumor that has very poor prognosis. Its growth is driven by cancer stem cell that has the potential to initiate and maintain the tumor. Targeting the cancer stem cell pool provides a novel approaches in treating glioblastoma. Therefore, understanding the regulatory network that governs the growth of glioblastoma stem cells is pivotal in controlling the tumor. We employed small molecule approach to probe the glioblastoma stem cell network. A previous screen in our lab showed a hit enrichment of neurotransmitter compounds when we screened unbiased bioactive library in mouse neural stem cells, therefore probing glioblastoma stem cells with neurotransmitter modulators is predicted to reveal novel targets. We interrogated all classes of neurotransmitters modulators containing 680 compounds in a series of glioblastoma neural stem cell lines (GNSC), human fetal neural stem cell lines (hfNSC) and BJ fibroblasts as a control. This screening approach allowed us to identify compounds that selectively inhibit the growth of GNSC and hfNSC. The primary screen identified 29 compounds that selectively inhibit the growth of hfNSC and GNSC when compared to BJ fibroblast at a screening concentration of 5uM. Further retesting at various doses validated 10 compounds that showed more than 8 fold selectivity. Compounds modulating dopaminergic, serotonergic and cholinergic pathways are enriched in hits suggesting a plausible role in neural stem cell network. Three out of ten compounds showed 2-6 fold more selective towards GNSC when compared to hfNSC and two of these compounds are dopamine receptor antagonists. In vivo validation of dopamine receptor antagonists in subcutaneous implanted tumor in NOD SCID gamma mouse showed 40% reduction in tumor mass. Furthermore, treated tumors have reduced clonogenic potential when compared to control by in vitro limiting dilution assay. This preliminary data suggests that dopam-

ine receptor antagonist reduces the tumor size by depleting the cancer stem cell pool. To further characterize the mechanism, we first confirmed the expression of dopamine receptor in hfNSC and GNSC lines. The preliminary knockdown experiment suggests that dopamine receptor inhibits the growth of GNSC. To elucidate the mechanism that confers GNSC selectivity, we performed phospho kinase profiling of 43 kinases. Interestingly, we observed differential response of GNSC and hfNSC towards dopamine receptor antagonists. The dopamine receptor antagonist decreases the phosphorylation of ERK1/2, Akt, TOR and p70S6 kinase in GNSC while there is no dramatic change in hfNSC. Dopamine receptors are known to activate ERK1/2 signaling. Selectivity of GNSC towards dopamine receptor antagonist may be partly conferred through ERK1/2 pathway, which is deregulated in glioblastoma.
In conclusion, we identified dopamine receptor antagonists that selectively inhibit the growth of glioblastoma stem cells both in vitro and in vivo. This selectivity may be partly conferred through ERK1/2 pathway, which is deregulated in GNSC or could be at receptor level with differential regulation or activity of dopamine receptors, which we are in process of studying. Dopamine receptor antagonist could potentially serve as a therapeutic agent in treating glioblastoma.

W-1024

TARGETING SELF-RENEWAL IN BRAIN TUMOR

Zhe Zhu[1], Muhammad Amir Khan[1], Jan Gronych[2], Olga Bernhardt[1], Andrey Korshunov[3], Bernhard Radlwimmer[2], Peter Lichter[2], Haikun Liu[1]
[A]Normal and Neoplastic CNS Stem Cells, [B]Division of Molecular Genetics, [C]Department of Neuropathology, [1]DKFZ, Heidelberg, Germany

Cancer stem cells (CSCs) have been suggested as potential therapeutic targets for treating malignant tumors, but the in vivo evidence supporting that is still in debate.
Here we established a mouse model that allows stem cell-specific gene targeting in unperturbed high-grade mouse brain tumor. Using a GFP reporter driven by the promoter of the nuclear receptor tailless (Tlx) in the transgenic mouse model, a neural stem cells (NSCs) specific transcription factor, we demonstrate that Tlx positive cells of primary brain tumors are mostly quiescent and they are strong candidates for brain tumor stem cells (BTSCs). After introducing a BTSC-specific knock-out of the tailless gene in primary mouse tumors, we observed a loss of self-renewal of BTSCs, which leads to significant prolongation of animal survival. Furthermore, we demonstrate that loss of Tlx in BTSCs leads to the induction of essential pathways mediating cell cycle arrest, cell death and neural differentiation. Additionally we show that Tlx is a prognostic marker in human high-grade brain tumors for worse survival.
Our study provides the first genetic evidence for targeting self-renewal pathway in established primary tumors and proves that BTSCs are suitable therapeutic targets, thus strongly supporting the CSCs hypothesis and the importance of therapeutic approaches targeting CSCs.

W-1025

POWER LAW GROWTH IN THE SELFRENEWAL OF HETEROGENEOUS GLIOMA STEM CELL POPULATION

Michiya Sugimori, YUMIKO HAYAKAWA, SATOSHI KURODA
Univ of Toyama/ Graduate School of Medicine and Pharmaceutical Sciences, Toyama, Japan

Cancer survives via recapitulation of cancer cell population beyond generations. Cancer stem cells (CSCs), which would be involved in the recapitulation process, exhibit heterogeneity resulting in a tumor development of a diverse cancer cell population. However, it still remains unclear whether and how CSC heterogeneity is maintained during tumor development. We employed a tumor neurosphere culture, where glioma stem cells undergo self-renewal and form clonal spheres while maintaining undifferentiated states in the methylcellulose containing medium. This study revealed, in detail, the self-renewal process of U87 human glioblastoma cell line derived glioma stem cell-like cell (GSCC) clones. The frequency distribution of cell number per clone, where the GSCC clones grew diversely, exhibited power-law pattern. Six repeated passage experiments suggested that diversity and power-law

in growth of GSCCs recapitulated beyond generations. Size-based fractionation experiments further showed that both small- and large-clone derived population regenerated populations following power-law pattern and the diversity in growth. These suggest a scale- and space-free code where heterogeneous GSCCs flexibly and reversibly change the growth traits. Thus, cancer repopulates beyond generations while maintaining CSC heterogeneity. Such plastic repopulation activity is involved in cancer exacerbation via metastasis and recurrence after radiation and chemotherapy. This study definitely suggests the importance of a power-law code in the CSC plasticity as a potential therapeutic target in cancer.

W-1026

ADDRESSING THE MECHANISMS OF TUMOR-TROPIC MIGRATION OF NEURAL STEM CELLS

Merve Karabörk, Tugba Bagci-Onder
School of Medicine, Koç University, Istanbul, Turkey

In addition to their applications in regenerative medicine, neural stem cells (NSCs) have recently been recognized as efficient delivery vehicles for therapeutics in a number of malignancies including brain tumors. The NSCs utilized in preclinical brain tumor models have been derived from various sources, mainly fetal CNS tissues and recently from differentiated from embryonic stem (ES) cells. While tumor-tropism of most NSC lines has been well documented, the mechanisms and the major molecules involved in such migratory behavior have been largely unexplored. To address these questions, in this study, we have compared the tumor-directed migration abilities of different NSC lines and characterized the molecular differences between the “highly migratory” and “stationary” NSCs. We have assessed the gene expression levels of cell motility genes (e.g. Rho family GTPases, cytoskeletal regulatory proteins, focal adhesion kinases), extracellular matrix and adhesion molecules (e.g. collagens, ECM proteases, integrins) and chemokine signaling components (e.g. chemokine receptors) in these cells. This survey yielded a number of candidate pathways that were highly active in the “highly migratory” NSCs. Exogenous activation of a subset of these pathways increased the migratory ability of stationary NSC lines. Our study provides a “tumor-tropism” signature of the NSCs and identifies key signaling molecules that can be exploited to further increase the penetration of NSCs into tumor parenchyma and therefore augment their therapeutic utility.

W-1027

TOWARDS THE SYSTEMATIC COMPARISON OF THE PROLIFERATIVE AND DIFFERENTIATION CAPACITY OF NEURAL STEM CELLS AND GLIOBLASTOMA STEM CELLS

Lionel Meyer[1], Aurore Fernandez[1], Christel Herold-Mende[2], Pierre Kehrli[3], Dominique Bagnard[1]
[1]MN3T, INSERM, Strasbourg, France, [2]Department of neurosurgery, University of Heidelberg, Heidelberg, Germany, [3]Department of neurosurgery, Hospital of Strasbourg Hautepierre, Strasbourg, France

One of the most striking discoveries of the past ten years in the field of cancer is the existence of cancer cell populations exhibiting stem cell like properties. These cells exhibit strong capacities of self-renewal, largely contributing to the progression of cancer. This has been particularly well established in glioblastoma, the most severe type of brain tumor. The molecular mechanisms controlling glioblastoma stem cells proliferation, migration and/or differentiation remains elusive. To address this question we developed a systematic approach in order to better understand these mechanisms in human GSC. Human biopsies are immediately processed to generate gliomapsheres using protocols similar to the one used for the production of neural stem cells and neurospheres. Spheres are subjected to immunocytochemistry characterization, sphere formation assay and differentiation assay. GSC behaviors are compared to the one of NSC.
Here, we will present such a comparison between NSC and NCH644 GSM previously generated from a patient biopsy. We found that both type of stem cells grow equally well giving rise to a rate of spheres production (RSP) of 4-6% over a period of 4 days (6.4% versus 6.2% p=0,9837). In this assay we used NSC between passages 5-10 and GSC between passages 20-32 in order to get stable RSP. Interestingly, we showed that both types of spheres were producing similar extracellular matrix molecules as demonstrated by equivalent staining for Laminin isoforms and

tenascin. When differentiating NSC and GSM on Poly-L-Ornithine, we found that spheres were producing similar proportion of MAP2 (neuronal marker) and GFAP (astrocyte marker) positive cells (20.2% versus 18.2%, p=0,5420 and 68.6% versus 66.7% p=0,5136 respectively). However, GSC showed increased percentage of O4 (oligodendrocyte marker) compare to NSC (19% versus 46% $p < 0.0001$). Interestingly, a various proportion of cells we co-stained for MAP2 and GFAP or GFAP and O4.
To address the migration and differentiation capacities of both types of spheres in culture conditions better reflecting the in vivo situation we are currently developing a slice overlay assay in which spheres are grown on the top of brain slices freshly prepared from postnatal mouse brain. While too preliminary for quantitative analysis our results suggest no regional preference for adhesion and migration. Further investigation will allow us to monitor differentiation when exposed to such in vivo-like conditions.

W-1028

MESENCHYMAL TRANSITION OF GLIOBLASTOMA STEM CELLS BY VEGF BLOCKADE

Kosuke Funato, Petrina Georgala, Rong Wang, Leif A. Droms, Viviane S. Tabar
Department of Neurosurgery, Memorial Sloan-Kettering Cancer Center, New York, NY, United States

Glioblastoma is the most malignant form of brain tumor and accounts for approximately 54% of all primary brain tumors. Despite huge clinical efforts, the median survival of glioblastoma patients has remained 1 year over the past decade. Since dysregulated angiogenesis and vascular malformation are one of the hallmark features of glioblastoma, the emerging antiangiogenic therapy including anti-VEGF antibody Bevacizumab (Avastin) has been considered as a promising therapy for glioblastoma. However, recent studies indicate that tumors become refractory to antiangiogenic therapy and the effect on overall survival is still controversial. Therefore, there is an urgent need to reveal the molecular and cellular mechanism of insensitivity to Bevacizumab.
It has been shown that glioblastoma stem cells (GSCs) are resistant to chemotherapy and radiotherapy, and contribute to recurrence. To investigate the role of GSCs in response to antiangiogenic therapy, we isolated CD133-positive GSCs from fresh tumor samples. Bevacizumab treatment of CD133-positive GSCs increased migration (14.9-fold, $p<0.01$) and expression of mesenchymal marker genes, such as PDGFRB and SMA (1.8 and 3.8-fold, respectively). Increased expression of mesenchymal genes was also observed in Bevacizumab-treated patients' samples and organotypic slices ("explants"), further indicating the mesenchymal transition by Bevacizumab treatment. We next isolated mesenchymal GSCs by the expression of PDGFRB. PDGFRB-positive GSCs had higher migratory and invasive capacity in comparison with PDGFRB-negative GSCs (1.4-fold, $p<0.004$). In addition, when injected into explants, PDGFRB-positive cells migrated to blood vessels, which is consistent with perivascular tumor invasion observed in Bevacizumab-treated patients. Finally, we found that PDGFRB-positive but not -negative GSCs differentiated to SMA-positive mural cells by the combination of Bevacizumab and TGF-β treatment (PDGFRB-positive, 33.6 ± 4.1 % *vs* negative, 3.8 ± 2.3 %, $p<0.003$), implying PDGFRB-positive GSCs share features with pericyte progenitor cells.
Taken together, our data indicates that Bevacizumab treatment induces the transition of CD133-positive GSCs to the mesenchymal lineage and PDGFRB can be used as a good marker for this transition.

W-1031

OCT4A ISOFORM ENHANCES AGRESSIVENESS OF MEDULLOBLASTOMA CELLS

Patrícia B G Silva, Carolina O. Rodini, Márcia Cristina T. Santos, Daniel Sanzio G. Cruz, Karina G. Oliveira, Oswaldo K. Okamoto
Biosciences Institute, São Paulo University, São Paulo, Brazil

Expression of OCT4 has been recently correlated with poor prognosis in different types of cancer including medulloblastoma, the most common and challenging type of embryonal tumor in childhood. However, direct evidence of OCT4 contribution to tumor aggressiveness is lacking. In this regard, defining the specific contribution of distinct OCT4 isoforms derived from alternative splicing is of particular importance. In this study, the effects of OCT4A overexpression on cell properties conferring a more aggressive tumor phenotype were determined in medulloblastoma.

Stable overexpression of OCT4A was performed by retroviral transduction of DAOY cells, followed by analyses of cell proliferation and tumorigenesis in vitro. Tumor cell proliferation was verified by population doubling level (PDL) assays, amount of viable cells based on MTT metabolism after 24, 48 and 72 hours, and cell cycle analysis by flow cytometry based on propidium iodide incorporation. In vitro tumorigenesis was accessed by colony formation in soft-agar after 15 days. Western blotting revealed that native DAOY cells express low levels of OCT4A protein. After retroviral transductions, cells displaying different levels of OCT4A overexpression were obtained. Cells with 2 increment were named OCT4A Low (lo) and cells with 4 increment were called OCT4A High (hi). Cells overexpressing OCT4A displayed shorter population doubling time than control cells (DAOY: 29,81±0.75h; Lo: 25.05±0.36h*, hi: 25.00± 0.38h*, *p<0.0001) evaluated after 50 generations in vitro. A similar increase in proliferation was also indicated by the MTT assay in all times tested (p<0.0001) and by cell cycle analysis, in which a higher proportion of OCT4A lo and hi cells were found to be in S and G2 phases, compared with the control cells (DAOY: 52.15±0.49%; lo: 60.76±0.43%*; hi: 58.76±1.11%*, *p<0.0001). Cells overexpressing OCT4A also generated higher amount of colonies when cultivated in soft agar, indicating enhanced anchorage-independent cell proliferation. Noteworthy, colonies of OCT4A overexpressing cells were larger than those of control cells (average diameter: DAOY: 71µm; lo: 241 µm; hi: 816 µm). These findings indicate that expression of OCT4A, an isoform with well characterized function in pluripotency and stem cell self-renewal, contribute to tumor aggressiveness and may be further explored as a potential indicator of poor prognosis in medulloblastoma.

W-1032

BMI1 REGULATES GLI ACETYLATION THROUGH CULLIN3 IN MEDULLOBLASTOMA STEM CELLS

Randy Van Ommeren[1], Chitra Venugopal[1], Branavan Manoranjan[1], Nicole McFarlane[1], Robin Hallett[1], John Hassell[1], Sandra Dunn[2], Sheila Singh[1]

[1]Biochemistry, McMaster University, Hamilton, ON, Canada, [2]Department of Pediatrics, University of British Columbia, Vancouver, ON, Canada

Constitutive activation of the sonic hedgehog (Shh) pathway in neural progenitor cells of the external granular layer (EGL) in the cerebellum is a recently described driver of brain tumour initiating cells (BTICs) in medulloblastoma (MB). We have shown that Shh pathway activation leads to increased production of Bmi1, a polycomb repressor which maintains 'stemness' in BTICs. Our current work demonstrates that up-regulation of Bmi1 by the Shh pathway leads to transcriptional repression of the E3 ligase, Cullin3 (Cul3). Cul3 has been shown to ubiquitinate histone deacetylase 1 (HDAC1), leading to increased acetylation of the Gli transcription factors which further activates the Shh pathway. Importantly, we utilize chromatin immunoprecipitation (ChIP) to demonstrate direct binding of Bmi1 to the Cul3 promoter exclusively in CD15+ MB stem cell populations.
This project is carried out with minimally cultured cells derived from primary patient tumours obtained through REB-approved protocols as well as with MB cell lines, Daoy and Med8a. Bmi1 and Cul3 expression is modulated using overexpression (OE) and inducible Tet-On® lentiviral-mediated shRNA knockdown (KD) vectors. mRNA and protein levels of Bmi1 and Cul3 following OE and KD are assessed by RT-PCR and western blotting (WB) respectively. Functional effects of OE and KD are analyzed by self-renewal and proliferation assays. Acetylation status of the Gli transcription factors is assessed by immunoprecipitation using anti-Gli antibodies and subsequent probing for acetyl-lysine on a WB. Bmi1 occupancy at the Cul3 promoter is elucidated through ChIP using Bmi1 antibody on CD15-sorted cells followed by PCR amplification of Cul3 promoter regions. Finally, the functional significance of the pathway in MB BTICs will be assessed using our in vivo BTIC xenograft model. Mice will undergo intracranial injection of MB cells carrying inducible Tet-On® shCul3 vector.
To date, ChIP analysis has demonstrated that Bmi1 is enriched at the Cul3 promoter in CD15+ stem/ progenitor cells though not in CD15- non-stem cells. Overexpression of Bmi1 has been shown by RT-PCR to cause reduced levels of Cul3 transcript, and an inverse result was observed for Bmi1 knockdown. Further, WB analysis shows that overexpression of Bmi1 leads to a reduction of Cul3 protein and a corresponding increase of HDAC1 in Med8a tumor spheres. Future functional elucidation of the pathway in vivo should demonstrate that Bmi1 KD produces less aggressive tumours by reducing Shh pathway activation in injected MB BTICs.

We suggest the existence of a positive feedback loop whereby Bmi1 can repress transcription of the Cul3 E3 ligase. This in turn leads to reduced ubiquitination of HDAC1 and increased Gli deacetylation which further activates Bmi1 transcription and drives the positive feedback system. We have previously demonstrated MB BTICs to be Shh-receptive cells that respond to ligand produced by bulk tumour. Our new findings suggest a novel mechanism whereby aggressive activation of the Shh pathway seen in neural progenitor cells of the EGL and MB BTICs may result in reduced expression of Cul3 as a result of increased repression by Bmi1. These findings pertain to both normal cerebellar development as well as cancer, and describe a novel pathway element responsible for producing a bona fide BTIC in pediatric MB. A more defined understanding of the pathways which promote a BTIC phenotype in the tumour cells of MB patients will provide novel drug targets for future therapies.

W-1033

DEMETHYLATION OF HISTONE 3 LYSINE 27 REGULATES THE SECRETION OF INFLAMMATORY FACTORS RESPONSIBLE FOR THE ROBUST TROPISM OF THERAPEUTIC NEURAL STEM CELLS

Michael E. Silva[1], Patrick Perrigue[1], Daniel Mota[2], Lauren Joseph[2], Joseph Najbauer[1], Karen S. Aboody[1]
[A]Neurosciences, [B]Eugene & Ruth Roberts Summer Academy, [1]City of Hope, Duarte, CA, United States

Neural stem cells (NSCs) have demonstrated their utility as a delivery vehicle for targeted cancer therapy and ischemic injury. However, defining what regulates the robust secretion of chemokines that attract NSC migration toward the tumor or sites of ischemia sites have not been clearly elucidated. Many of these secreted factors have been associated with recruiting a host immune response. JMJD3, a histone 3 lysine 27 demethylase, has been shown to be involved in wound healing and tumorigenesis. Here, we investigate the epigenetic role JMJD3 has in recruiting NSC migration towards human glioma cell lines and senescent-induced fibroblasts. To test our hypothesis of stem cell migration towards glioma or human senescent-induced fibroblasts, we began by using a Boyden chamber-based cell migration assay. Our data indicates that JMJD3 is both necessary and sufficient to activate the robust expression of chemokines that may be responsible for significantly increasing the migration of our therapeutic NSC line (HB1.F3.CD) nearly 17-fold, as well as mesenchymal stem cells (MSCs), towards conditioned media collected from human glioma cell lines as well as senescent human dermal fibroblasts. We further show that the expression of these specific inflammatory factors is both necessary and sufficient through an epigenetic mechanism involving JMDJ3 using ChIP analysis. We also used a human cytokine antibody array, rt-PCR and FACS analysis to identify multiple factors and their corresponding receptors that have previously been shown to be necessary and sufficient in directing NSC migration. In vivo studies are in progress. Collectively, these data will help expand our understanding of the tumor biology involved in stem cell migration and may lead to the increased efficacy of applying NSCs to target and deliver therapeutic agents to tumor foci for cancer therapy as well as other diseases associated with chronic inflammation.

W-1034

NANOG EXPRESSION IN HUMAN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

Marta Cañamero[1], Manuel Rodriguez-Justo[3], Manuel Serrano[2], Daniela Piazzolla[2]
[A]Histopathology, [B]Tumor Suppression Group, [1]CNIO, Madrid, Spain, [2]Pathology, Department of Researh Pathology, University College Hospital, London, United Kingdom

Esophageal carcinoma is the eighth most common cancer and the sixth leading cause of death from cancer worldwide and one of the most common malignant diseases in China. It can be subdivided in two major histological types: Adenocarcinoma (EAC) and squamous cell carcinoma (ESCC). Unfortunately the majority of ESCC patients have locally advanced or metastatic disease at the time of diagnosis and the overall 5-year survival is poor. Screening and early diagnosis of esophageal cancer remains a challenge in spite of introduction of new endoscopic techniques. Although the identification of biomarkers in diagnosis and tumour progression and, the identification of

new therapeutic targets is an area of active research; no biomarkers have been translated to routine clinical practice yet.
NANOG is a homeobox transcription factor that has been identified as a key player in the establishment of embryonic stem cells (ES cells) identity and functions in concert with other transcription factors such as SOX2.
There are several studies reporting NANOG expression in a variety of human tumors, such as gonadal germ cell tumors, central nervous system germinomas, medulloblastoma, glioma and epithelial tumors.
Here we investigate NANOG expression in formaline-fixed paraffin-embedded sections of 29 patients (18 males and 11 females) of ESSC by immunohistochemistry and we discovered that NANOG is expressed in 100% of the cases with a perinuclear or nuclear pattern. In addition, we compared NANOG expression with its stem partner SOX2, that has been recently described to be amplified in human ESCC, finding a correlation between their expressions. These data suggest that NANOG could be a possible new biomarker for use in ESCC diagnosis. Its potential as a predictive and behavioral marker would require further studies.
We are currently carrying out a parallel analysis of NANOG expression in a variety of ESSC cell lines.

W-1035

STEMNESS PROTEIN LEVEL: A SURROGATE MARKER FOR TAMOXIFEN RESISTANCE BREAST CANCER

Issam Hussain, Khalid Arif, Carol Rea, Mohamed El-Sheemy
School of Life Science, Univ of Lincoln, Lincoln, United Kingdom

Introduction: It is well documented that using antihormonal therapy with tamoxifen significantly improves the survival rate of women with oestrogen-receptor positive (ER+) breast tumours. However, the development of resistance to tamoxifen as a primary therapy and disease reoccurrence limits its clinical benefit. This therapeutic failure has revived interest in the breast cancer stem cell (BCSC), since stem cells were reported to be resistant to antihormonal therapy. Stemness" genes expression increases in undifferentiated cells. However, the role of their expression in breast cancer is still poorly defined. The aim of this study was to examine the role of stemness proteins in breast cancer cell lines and anti-hormonal therapy resistance.
Method: MCF-7, MCF-7/tamoxifen resistant (TAMR) and MDA-MB238 cells were cultured, with relevant hormonal therapy to calculate ED50 and incubated in 5% CO2 incubator at 37°C. Quantitative and qualitative immunophenotyping of cells was achieved using fluorescein isothiocyanate (FITC) labelled antibodies reactive with NANOG, SOX2, OCT4 and CD44.
Result: MDA-MB238 cells showed significant expression of NANOG (70%), CD44 (85%) SOX2 (68%) and OCT3-4 (50%) compared with MCF-7(12%, 4%, 15% and 10% respectively). However the MCF-7 TAMR demonstrated lower expression of NANOG (45%), CD44 (60%), SOX2 (60%) and OCT3/4 (55%) compared with MDA-MB238.
Conclusion: Stemness proteins are highly expressed in breast cancer cells resistant to tamoxifen therapy. There is association between the expression of Stemness proteins level and the development and progression of breast cancer resistance to tamoxifen therapy

W-1036

TRANSIENT EXPRESSION OF REPROGRAMMING FACTOR IN VIVO LEADS TO TUMOR DEVELOPMENT

Kotaro Onishi[1], katsunori Semi[1], Akito Tanaka[1], Takuya Yamamoto[1], Masahito Shimizu[2], Hisataka Moriwaki[2], Shinya Yamanaka[1], Knut Woltjen[1], Yasuhiro Yamada[1]
[1]Kyoto Univ CiRA, Kyoto City, Japan, [2]Gastroenterology, Gifu Univ graduate school of medicine, Gifu city, Japan

Induction of 4 transcription factors, Oct3/4, Sox2, Klf4, Myc in differentiated somatic cells has yielded induced pluripotent stem cells (iPSCs). Although generation of iPSCs does not required changes in genomic sequence, iPSC acquired indefinite growth potential: the key characteristic shared with cancer cells. In this study, we generated a reprogrammable mouse in which reprogramming factor expression can be controlled temporally in vivo by the treatment of Doxycycline (Dox).

Induction of reprogramming factors in vivo caused active cell proliferations and a rapid expansion of dysplastic lesions in the epithelial tissues. Prolonged expression of reprogramming factors caused invasive growth of dysplastic cells, leading to cancer development in various organs.
Following longer period of reprogramming factors induction, we observed dysplastic proliferating cells in the kidney, which continued neoplastic growth even after Dox withdrawal. Histological analysis revealed that these Dox-independent kidney tumors resemble Wilms' tumor, a common pediatric kidney cancer. In addition, microarray analysis demonstrated that many upregulated genes in the Dox-independent kidney tumors were similarly upregulated in Wilms' tumor.
Our results suggest that aspects of cellular reprogramming may be involved in the development of pediatric cancers.

W-1037

DUAL EFFECTS OF HUMAN ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS ON NF-κB PATHWAY IN TUMOR CELL GROWTH OF A549 AND HT-29.

Jung Joo RHYU, Euna KWON, Byeong Cheol KANG
Dep't of Experimental Animal Research, Seoul National University Hospital, Seoul, Korea, Republic of

Human adipose tissue-derived mesenchymal stem cells (hATMSCs) have a great potential as therapies for various diseases and regenerative medicine. Recently, emerging evidence suggests that human stem cells have both promoting and inhibitory effects on tumor growth. However, little is known about factors that make these contradicting results, and the therapeutic use of hATMSCs should be cautious under malignant conditions. The purpose of this study is to determine in which tumor environment hATMSCs have tumor supporting effects or suppressing effects and to understand the underlying mechanism.
We investigated the effect of hATMSCs on growth of 6 different types of tumor cell lines *in vivo*. A-375, A-431, A549, NCI-N87, HT-29 and Capan-1 cell lines were implanted into athymic nude mice. And the each tumor xenograft mice were treated with intratumoral injection of hATMSCs (1×10^5 cells/mouse) once a week for 8 weeks or with PBS injection for control groups instead. Tumor volumes and weights were measured. The results showed that hATMSCs have an inhibitory effect on tumor growth of A549 and Capan-1, but a promoting effect on HT-29. We focused on A549 and HT-29 tumor and performed further protein analysis. The expression level of tumor-related proteins such as NF-κB/p65, Akt, JAK3, STAT3 and β-Catenin was analyzed by western blotting. The results showed there is an obvious correlation between the tumor growth and the expression level of NF-κB/p65. We also confirmed the similar effect of hATMSCs *in vitro*. When A549 or HT-29 tumor cells were co-cultured directly or indirectly with hATMSCs respectively, the proliferation rate of A549 was decreased but of HT-29 was enhanced.
In this study, we demonstrated the dual effects of hATMSCs, which is inhibiting effect on A549 and promoting effect on HT-29, and the correlation between the tumor growth and the expression level of NF-κB. Despite the controversies concerning the relationships between the MSCs and the tumors, the application of stem cells in cancer therapy or regenerative medicine has broad prospective. Therefore, we consider that it is required further research to understand the interactions between stem cells and various types of tumors and those understandings will provide a new clue for stem cell therapy.

W-1041

EVIDENCE THAT BREAST CANCER STEM AND PROGENITOR CELLS MODIFY TUMOR GLUCOSE METABOLISM IN RESPONSE TO A HYPOXIC MICROENVIRONMENT

Nicole Gordon, Robynn Schillace, Rodney Pommier, Steven O'Neill, Patrick Muller, Arpana Naik, Robert O'Rourke, Juliana Hansen, Jennifer Alabran, Cory Donovan, SuEllen Pommier
Oregon Health & Science University, Portland, OR, United States

Background: Increases in hypoxia response gene expression and aberrant glucose metabolism are observed in breast cancers. This may endow tumors with the plasticity needed to sustain growth in avascular tissues. The ob-

jective of this study was to determine if adaptive changes are mediated through breast cancer stem/progenitor cells (BCSC) and whether this is determined by BCSC oncogene mutations.
Methods: From 15 invasive ductal carcinomas and 11 normal breast reduction specimens, cells variably expressing breast stem/progenitor cell surface markers CD44, CD49f, CD24 (lin-CD31 and CD45) were collected by cell sorting. RNA/cDNA and wgDNA were collected from whole tumor tissues, benign breast tissues and sorted cells. Genomic mutations were examined at 430 sites of 30 oncogenes (Sequenome MassARRAY). Expression of 36 hypoxia and glucose metabolism-related genes (HYPOX-GluMet) was measured by qRT-PCR (Life Technologies microfluidic cards). In addition, a subset of ex vivo benign and malignant breast surgical specimens were grown overnight in hypoxic (1%) or normoxic (21%) conditions prior to cell sorting. Absolute delta Cts in gene expression were calculated and significance was determined by Student's t-test and ANOVA ($p<0.05$).
Results: Compared to benign breast tissue, tumor specimens showed significant increased HIF1a gene expression. BCSC compared to benign stem cells (SC) showed significant changes in HYPOX-GluMet. Specifically, BCSC lin-CD49f+CD24+ and lin-CD49-CD24+ showed decreased SLC2A1 (GLUT1),and lin-CD49f+CD24- showed decreased SLC2A3 (GLUT3), HK2, SLC16A3, G6PD, SOD2 and BNIP3. Benign SC, and breast tissues had few significant inter-group differences in HYPOX-GluMet. In contrast, 3 of 4 BCSC showed significant inter-group variability and decreased HYPOX-GluMet compared to tumors. Lin-CD49f-CD24- BCSC had higher HYPOX-GluMet gene expression than all other BCSC and were similar to tumors. After 24 hours of normoxic or hypoxic exposure, lin-CD49f-CD24+ and lin-CD49f+CD24+ cells decreased in number by 85% and 62% respectively in benign tissues and 82% and 52% in tumors. Lin-CD49f+CD24- and lin-CD49f-CD24- cells increased by 44% and 5.7% respectively in benign tissues and 45% and 2.6% in tumors. HYPOX-GluMet gene expression was influenced by normoxic and hypoxic conditions in SC and BCSC. Hypoxia decreased HYPOX-GluMet in benign SC, while BCSC showed population-specific CD44 expression increased under hypoxia in both SC and BCSC. Four of 15 tumors had PIK3CA mutations, 3 had SMO, and 2 had PIK3CA and SMO mutations (4 previously reported). HYPOX-GluMet did not correlate with the presence of any specific oncologic mutation.
Conclusions: Changes in HYPOX-GluMet occur following malignant conversion of benign SC to BCSC. These changes are not dependent on PIK3CA or SMO mutations. Gene expression profiles indicate that BCSC lin-CD49f+CD24- cells become significantly metabolically less active in tumors. Lin-CD49f-CD24- cells appear to be more committed-progenitor cells, are highly metabolically active, very proliferative and most reflect the HYPOX-GluMet status of the tumors. BCSC are able to adapt to anaerobic and aerobic microenvironments through changes in cell numbers and transcriptional changes. These abilities of BCSC may help ensure that tumors are able to survive under the variable conditions encountered during progressive stages of tumor growth.

W-1042

THE PLURIPOTENCY FACTOR NANOG IS EXPRESSED IN ADULT TISSUES AND INDUCES HYPERPLASIA WHEN OVER-EXPRESSED

Daniela Piazzolla[1], Adelaida R. Palla[1], Marta Cañamero[2], Gonzalo Gómez-López[3], Orlando Domínguez[4], Sagrario Ortega[5], Beatriz Fernandez-Tresguerres[6], Agustin F. Fernandez[7], Mario F. Fraga[7], Miguel Manzanares[6], Konrad Hochedlinger[8], Manuel Serrano[1]

[A]Tumor Suppression Group, [B]Histopathology Unit, [C]Bioinformatics Unit, [D]Genomics Unit, [E]Transgenic Mice Core Unit, [1]Spanish National Cancer Research Centre (CNIO), Madrid, Spain, [2]Department of Cardiovascular Developmental Biology, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain, [3]Cancer Epigenetics Laboratory, Instituto Universitario de Oncología del Principado de Asturias (IUOPA), HUCA, Universidad de Oviedo, Oviedo, Spain, [4]Harvard University and Harvard Medical School/ Massachusetts General Hospital Cancer Center and Center for Regenerative Medicine/ Harvard Stem Cell Institute, Cambridge/ Boston, MA, United States

NANOG is a key transcription factor for the acquisition and maintenance of embryonic stem cells pluripotency. Deletion of *Nanog* in mice causes early embryonic lethality due to the absence of the epiblast and downregulation of *Nanog* in both mouse and human embryonic stem cells (ESCs) predisposes cells toward differentiation. Conversely, over-expression of NANOG renders both mouse and human ESCs cytokine- and feeder layer-independent. NANOG

has been shown to be highly expressed in the inner cell mass, in derived ESCs, in embryonic germ (EG) cells and in embryonal carcinoma (EC) cells. Contrary to the very well-established role of NANOG in regulating embryonic stemness, the possible function of this transcription factor in adult tissues is still unexplored. Interestingly, recent studies have reported the expression of NANOG in some specific human tumors, suggesting that this stemness transcription factor could also play a role in cancer.
In order to investigate the presence of NANOG in adult tissues we performed a systematic analysis of the patterns of NANOG expression by immunohistochemistry and *in situ* hybridization in a variety of mouse tissues and we extended this analysis to tumors of different origins. Our data indicates that NANOG is expressed in specific mouse tissues and tumors. These findings were further supported by the analysis of *Nanog* promoter methylation status.
Based on these results, we explored the functions of NANOG in adult life by characterizing a transgenic mouse model where NANOG expression is ubiquitously inducible. We discovered that over-expression of NANOG induces hyperplasia and dysplasia *in vivo* associated with hyperproliferation and increased DNA damage.
We are currently trying to dissect the molecular mechanism underlying NANOG's ability to induce proliferation.

W-1043

P53 LOSS OF HETEROZYGOSITY OCCURS IN THE BONE MARROW AND GIVES RISE TO TUMOR INITIATING MESENCHYMAL STEM CELLS

Yoav Shetzer, sivan kagan
Weizmann institute of science, rehovot, Israel

Cells heterozygous for p53 mutation undergo loss of the WT p53 allele by a process termed loss of heterozygosity (p53LOH) which leads to cancer development. The aim of this study was to challenge the hypothesis that p53LOH occurring in adult stem cells is an initiating event leading to cancer. Interestingly, p53LOH already occurred in the bone marrow (BM) of heterozygous p53 mutant mice, where it may either involve the loss of WT or mutant p53 allele, with a preference of losing the mutant allele. However, *in-vitro* established mesenchymal stem cells (MSCs) isolated from BM of heterozygous p53 adult mice, underwent exclusively WT p53LOH and were able to generate tumors *in-vivo*. WT p53LOH was facilitated with age. Analysis of the gene expression profile along the LOH process in heterozygous p53 MSCs has indicated Homologous Recombination (HR) DNA repair pathway, which is known to be p53 transcription-independent, was up-regulated. Likewise, the expression of *Smarca1,* a chromatin remodeler, was induced. Importantly, *Smarca1* up-regulation was observed during p53LOH process also in Mouse Embryonic Fibroblasts (MEFs). Our results suggest that p53LOH process, occurring in adult stem cells such as MSCs, would raise their risk to become tumor initiating cells.

W-1044

APOPTOSIS INDUCED BY PGC-1β IN BREAST CANCER CELLS IS MEDIATED BY MTOR PATHWAY

Libin Wang[1], Yinxue Yang[2], Haibing Ma[2], Heng Fan[2], Yongzhao Zhu[2], Yaqing He[2], Yukui Li[2]
[1]Ningxia Human Stem Cell Res Inst,General Hospital of Ningxia Medical university, Yinchuan, China, [2]Ningxia Human Stem Cell Res Inst, Yinchuan, China

The peroxisome proliferator-activated receptor-gamma (PPAR-gamma) coactivator-1β(PGC-1β) is a well-established regulator of the mitochondrial biogenesis. However, the underlying mechanism of PGC-1β action remains elusive. This study reveals that knockdown of endogenous PGC-1β by shRNA leads to a decrease in the expression of mTOR pathway-related genes in MDA-MB-231 cells. After knockdown of PGC-1β, phosphorylation of AMP-activated protein kinase (AMPK), phosphorylation of Rictor on Thr1135, Raptor and S6 protein is inhibited. However, Akt phosphorylation on Ser473 is upregulated and cell apoptosis occurs. In particular, we demonstrate that the level of PGC-1β and mTOR correlated with overall mitochondrial activity. These results provide new evidence that cell apoptosis is orchestrated by the balance between several signaling pathways, and that PGC-1β take part in these events in breast cancer cells mediated by mTOR signal pathway.

W-1045

HISTONE3 LYSINE9 DI-METHYLATION AND DNA HYPOMETHYLATION DURING THE PRE-NEOPLASTIC STAGE OF EXPERIMENTAL MOUSE GLIOMA

Monica Nistér[1], Xiaobing He[1], Mohsen Karimi[2], Mingqi Qu[1], Anna Hedrén[1], Tomas J. Ekström[2]
[A]Department of Oncology Pathology, [B]Department of Clinical Neuroscience, [1]Karolinska Institutet, Stockholm, Sweden

Glioblastoma is one of the most malignant human tumors. To find new strategies for therapy, we need more knowledge on the essential molecular changes during the early stages of glioma development. We have previously generated a transgenic mouse with forced expression of platelet-derived growth factor B (PDGFB) in astrocytic cells of the brain and crossed these mice onto a p53null background, thereby mimicking the genotype of human secondary glioblastoma. The hGFAPpPDGFB/Trp53 null (B+p53-/-) mice develop glioblastoma-like brain tumors at a high frequency in adulthood. The present work reveals epigenetic changes in the mouse brain tumors and in neural stem/progenitor cells during the pre-neoplastic stage. DNA hypomethylation and elevated Histone3 Lysine9 di-methylation (H3K9Me2) were detected in the tumors by immuno-histochemical stainings. During the pre-neoplastic stage, global DNA hypomethylation was observed in samples of the adult frontal brain lateral ventricular wall (LVW) using the Luminometric Methylation Assay (LUMA), but could not be observed in whole brain samples. Furthermore, elevated H3K9Me2 levels were observed in neurosphere cultures from the B+p53-/- pre-neoplastic brain but not in wild type (WT) neurospheres using immuno-blot analysis. The H3K9Me2 level was also higher in brain tumor spheres compared to neurospheres derived from the same mouse. We propose that epigenetically disturbed neural stem/progenitor cells might be the early targets of neoplastic transformation in the brain.

W-1046

OVARIAN CANCER STEM CELLS: PREDICTOR FOR THERAPY IN ADVANCED CHEMO-RESISTANT OVARIAN CANCER.

Soma Mukhopadhyay, Prattusha Sengupta, Sudeshna Gangopadhyay, Ujjal kanti Roy, Ashis Mukhopadhyay
Netaji Subhas Chandra Bose Cancer Research Inst, Kolkata, India

Background: Invasive and mesenchymal property of Ovarian Cancer Stem Cells (OCSCs) with CD44+/CD133+ has made them promising target for targeted treatment. Chemotherapy treatment uses medicine to weaken and destroy cancer cells in body, including cells at original cancer site and any cancer cell that may have spread to another part of body. Chemotherapeutic drugs for advanced chemo-resistant ovarian cancer are yet to be well defined. Combination of drugs is also not fully known. Our objective is to define chemotherapeutic drugs and its action in OCSC which is the major reason for chemo-resistance in case of advanced chemo-resistant ovarian cancer patients.
Methods: A total of twenty eight biopsy proven advanced chemo-resistant ovarian cancer patients in the age group of 22-36 years were selected randomly and tested for CD44/CD133 via flowcytometry. Isolated OCSCs were cultured for ex vivo drug sensitivity towards Platinum, Anthracyclin, Docetaxel, Rapamycin, Sunitinib, Sorafenib and Gefitinib. Correlation was drawn between cell differentiations, percentage of stem cells and drug response. Accordingly chemotherapy was designed for a particular patient.
Results: We detected OCSCs in 89.3% of cases. Among positive samples ex vivo drug sensitivity was seen in 5(20%) to Rapamycin, 1(4%) to Sunitinib, 1(4%) to Sorafenib, 1(4%) to Gefitinib, 4(16%) to Platinum, 1(4%) to Anthracyclin, 1(4%) to Docetaxel and rest showed no sensitivity to any drug.
Conclusions: Thus primary aim to target OCSCs at onset of tumors in ovarian cancer patients to control metastasis and relapse of disease was somewhat obtained. Most interestingly, we found that the chemotherapeutic drugs which were less prescribed for ovarian cancer showed greater sensitivity in comparison to the widely used ones. We like to do Animal model study followed by Phase I, II and III Human Clinical Trial to establish our hypothesis for better management of chemo-resistant ovarian cancer.

Chromatin in Stem Cells

W-1051

AUTOSOMAL SPREADING OF XIST REVISITED.

Agnese Loda, Joost Gribnau
Reproduction and Development, Erasmus University Medical Centre, Rotterdam, Netherlands

In mammals, dosage compensation of sex chromosomal genes between females (XX) and males (XY) is achieved through X chromosome inactivation (XCI). This epigenetic phenomenon involves non-coding RNAs, antisense transcription and histone modifications to distinguish two genetically identical X chromosomes as active (Xa) and silent (Xi) entities within the same nucleus. At the onset of XCI, the X-linked non-coding Xist gene is transcriptionally upregulated on the future Xi. Xist RNA coats the Xi in cis triggering chromosome wide transcriptional inactivation. Although Xist is the major player of the process the molecular mechanism by which Xist RNA spreads along the entire length of the X chromosome is an intriguing open question. Notably, many X to autosome translocations show either inefficient Xist spreading or incomplete inactivation of the autosomal translocated material, suggesting a sequence specific model for Xist spreading in cis (White et al. 1998, Popova et al. 2006). In this context, LINE repetitive elements, which are enriched on X chromosome relative to autosomes have been implicated to confer X-chromosome specificity (Lyon et al. 1998). Nevertheless, we hypothesize that the lack of autosomal inactivation observed in cells with X:autosome translocations reflects negative selection against those cells in which Xist spreading was efficient enough to transcriptionally inactivate the autosomal fraction of the translocation product thus leading to lethal aneuploidy. To further investigate this hypothesis we generated (41, XX dup12) ES cell lines with a tetracycline-responsive Xist transgene integrated on chromosome X, 12, or other autosomes. The use of polymorphic ES cell lines allows us to evaluate the degree of gene inactivation by performing allele specific expression analysis. Silencing of one of the three chromosome 12's in these (41, XX dup12) ES cell lines will not be lethal similar to silencing of one X chromosome in female cells. This may contrast with ES cell lines harbouring transgene integrations on other autosomes. Therefore, comparison of Xist mediated silencing in these different ES cell lines will indicate whether aneuploidy or the LINE density explains reduced Xist spreading on autosomes.

W-1052

REACTIVATION OF X CHROMOSOME UPON REPROGRAMMING LEADS TO CHANGES IN THE REPLICATION PATTERN AND 5hMC ACCUMULATION

Alexandra Bogomazova, Alexandra Panova, Evgueny Nekrasov, Sergey Kiselev, **Maria Lagarkova**
Vavilov Institute of General Genetics, Moscow, Russian Federation

Once set, the inactive status of the X chromosome in female somatic cells is preserved throughout subsequent cell divisions. The inactive status of the X chromosome is characterized by many features, including late replication. In contrast to induced pluripotent stem cells (iPSCs) in mice, the X chromosome in human female iPSCs usually remains inactive after reprogramming of somatic cells to the pluripotent state, although recent studies point to the possibility of reactivation of the X chromosome. Here, we demonstrated that, during reprogramming, the inactive X chromosome can switch from asynchronous to synchronous replication, with restoration of the transcription of previously silenced genes. This process is accompanied by accumulation of a new epigenetic mark or intermediate of the DNA demethylation pathway, 5-hydroxymethylcytosine (5hmC), on the activated X chromosome. Our results indicate that the active status of the X chromosome is better confirmed by synchronous replication and the re-appearance of 5hmC, rather than by appearance of histone marks of active chromatin, removal of histone marks of inactive chromatin, or an absence of XIST coating.

W-1053

FMR1 METHYLATION DYNAMICS IN FRAGILE X AFFECTED HUMAN EMBRYONIC STEM CELL LINES

Rachel Eiges[1], Michal Avitzour[1], Hagar Mor-Shaked[1], Shira Yanovsky-Dagan[1], Gheona Altarescu[2], Talia Eldar-Geva[3], Ephrat Levy-Lahad[2], Paul Renbaum[2], Silvina Epsztejn-Litman[1]

[A]Stem Cell Research Laboratory, [B]Medical Genetics Institute, [C]IVF Unit, [1]Shaare Zedek Medical Center, Jerusalem, Israel

Fragile X syndrome (FXS) is the most common non-chromosomal form of inherited mental retardation. It is inherited as an X-linked trait and is caused by a CGG trinucleotide repeat expansion (>200 CGGs) in the 5'-untranslated region of the FMR1 gene. The CGG repeat region is embedded within a ~ 1.1kb intragenic CpG island (chrX: 146993092-146994200), spanning from the promoter sequence to intron 1 of FMR1. As a CpG island, this region typically remains free of DNA methylation and is rich in histone modifications associated with transcriptional active chromatin. Yet, expansion of repeat copy number to over 200 CGGs leads to local acquirement of aberrant DNA methylation and repressive histone modifications by an unknown mechanism, resulting in the silencing of FMR1 gene in FXS patients.

Here we report on altogether 9 different HESC lines bearing pathologic expansions larger than 200 copies at the FMR1 gene. This collection of undifferentiated FXS cells, which is the largest to the best of our knowledge, comprises 5 male and 4 female cell lines. Taking advantage of this unique set of cells, we show that aberrant CpG methylation is already present at the 5'-end of FMR1 in the majority of FXS HESC lines (7/9), ranging from 20% to nearly 65%, and is steady over time in culture. Methylation levels are consistently higher downstream *versus* upstream to the CGG repeats, and are correlated with decrease in FMR1 mRNA levels and abnormal acquirement of histone modifications associated with inactive chromatin. Furthermore, by characterizing methylation levels following *in vitro* (embryoid body; EB) and *in vivo* (teratoma) differentiation we show that methylation, when set, can be enhanced by differentiation. This cannot be accounted for by the loss of CTCF binding next to the CGGs, as formerly suggested, since no enrichment for CTCF is detected within this region in wild type and affected HESC lines by chromatin immunoprecipitation experiments. Taken together, the findings of this study demonstrate that (1) aberrant methylation is frequently present near the CGGs in the undifferentiated state (2) differentiation enhances, rather than triggers abnormal CpG methylation at the FMR1 locus; and (3) CTCF does not appear to be involved in protecting the FMR1 region from heterochromatin induction during early embryo development.

Our FXS HESCs provide an exceptional opportunity to follow the dynamics of CpG methylation before complete FMR1 gene silencing is achieved. Furthermore, on a more general level, these HESCs also comprise a powerful model system by which one may investigate how CpG islands lose protection from DNA methylation.

W-1054

CHD7 REGULATES NEUROGENIC CELL FATE DECISIONS IN MURINE HIPPOCAMPAL ADULT NEURAL STEM CELLS

Kieran M. Jones[1], Pete Scambler[2], M. Albert Basson[1]

[1]Craniofacial Development and Stem Cell Biology, King's College London, London, United Kingdom, [2]Institute of Child Health, University College London, London, United Kingdom

The adult mammalian brain possesses the remarkable capacity to generate new neurons and glia throughout life. This process depends on the presence of neural stem cells (NSCs), which reside in two distinct regions of the forebrain: the subventricular zone of the walls of the lateral ventricles, and the subgranular zone of the dentate gyrus of the hippocampus (SGZ). Hippocampal neurogenesis has been shown to play an important role in certain types of learning, memory formation, and pattern separation. Chromatin remodelling can engage or maintain particular genetic programs and has been shown to play a role in the differentiation and self-renewal of embryonic and postnatal neural precursor cells, mainly affecting the self-renewal of precursor cells. Therefore, an understanding of the role of chromatin remodelling in adult NSCs and their progeny could provide insights into the regulation stem cell maintenance and daughter cell differentiation.

Recently, the chromatin remodelling factor Chromodomain-helicase-DNA-binding protein 7 (CHD7) has been shown to interact with SOX2, a NSC marker, and can also bind to regulatory regions in the vicinity of genes involved in NSC lineage progression. Here, we show that CHD7 is expressed in MASH1+ neuronally-restricted transit-ampli-

fying cells in the SGZ niche . *In vitro* assays using *Nestin-Cre; Chd7$^{flox/flox}$* NSC cultures demonstrate normal growth and normal astrocytic differentiation of Chd7-depleted NSCs, but mutant cells have an impaired capacity to form MASH1+ cells. Using a conditional *GLAST-CreER^{T2}; Chd7$^{flox/flox}$* mouse line to specifically delete *Chd7* in the adult NSC lineage and astrocytes, we show that a short-term deletion of *Chd7* results in a decrease in the number of MASH1+ transit amplifying cells and DCX+ neuroblasts, with a reciprocal increase in the number of astrocytes in the dentate gyrus. We demonstrate that deletion of *Chd7* from the adult NSC lineage also leads to a loss of NSC quiescence in the SGZ, possibly due to loss of quiescent ques from transit-amplifying cells and altered Notch signalling. Using BrdU-labelling studies we find that after a long-term deletion of *Chd7* from the adult NSC lineage fewer DCX+ progenitors and NeuN+ differentiated neurons are formed and a greater number of GFAP+ astrocytes are generated compared to controls. These findings suggest that the loss of NSC quiescence results in the depletion of neurogenic potential over time.
We conclude that CHD7 is required for the generation and / or maintenance of neuronally-restricted transit amplifying cells in the adult dentate gyrus, and loss of *Chd7* results in astrocytic differentiation instead of neuronal differentiation with a concurrent loss of NSC quiescence. These experiments identify a role for chromatin remodelling in regulating daughter cell differentiation and stem cell maintenance in a neurogenic niche in the adult mammalian brain.

W-1055

HMGN1 MODULATES NUCLEOSOME OCCUPANCY AND DNASEI HYPERSENSITIVITY AT THE CPG ISLAND PROMOTERS OF EMBRYONIC STEM CELLS

Tao Deng[1], Iris Zhu[1], Shaofei Zhang[1], Fenfei Leng[2], Srujana Cherukuri[1], Loren Hansen[1], Leonardo Mariño-Ramírez[1], Eran Meshorer[3], David Landsman[1], Michael Bustin[1]
[1]NIH, Bethesda, MD, United States, [2]Florida International University, Miami, FL, United States, [3]The Hebrew University of Jerusalem, Jerusalem, Israel

Chromatin structure plays a key role in gene expression and embryonic differentiation; however the factors that determine the organization of regulatory sites in chromatin are not fully known. Here we show that HMGN1, a nucleosome binding protein ubiquitously expressed in vertebrate cells, preferentially binds to CpG islands-containing promoters, and affects the organization of nucleosomes, DNaseI hypersensitivity, and transcriptional profile of mouse embryonic stem cells and neural progenitors. Loss of HMGN1 alters the organization of an unstable nucleosome at the transcription start sites, reduces the number of DNaseI hypersensitive sites genome wide, and decreases the number of Nestin-positive neural progenitors in the SVZ region of mouse brain. Our study provides insights into the mechanisms whereby an architectural nucleosome binding protein affects chromatin structure during embryonic stem cell differentiation.

W-1056

ROLE OF NUCLEOSOME POSITIONING IN HUMAN EMBRYONIC STEM CELLS

Puya G. Yazdi, Brian Pedersen, Jared Taylor, Ping H. Wang
Medicine, University of California at Irvine, Irvine, CA, United States

Human embryonic stem cells (hESCs) hold great potential to radically alter human medicine. This potential is a result of their pluripotent potential which is a direct consequence of their epigenomic state. The nucleosome, the fundamental repeating unit of chromatin, plays a pivotal role in influencing epigenetics by two major mechanisms: first by their positioning in the genome, thereby allowing access to DNA from certain transcription factors while covering binding motifs of others; second, nucleosomes can undergo extensive post-translational modifications on their histone tails, which has been demonstrated to effect transcription. In order to elucidate the role nucleosome positioning plays in hESC biology we have generated roughly 60x fold coverage of the human genome by illumina paired-end sequencing of mnase-digested H1 embryonic stem cell mononucleosomes. Our ultra

deep sequencing has begun to demonstrate the role nucleosome positioning plays in the epigenetics of stem cells. Our results indicate that not only are certain underlying DNA sequences enriched within a nucleosome, but that methylated cytosines are also greatly enriched within nucleosomes. Additionally, we have also studied the association of '5-hydroxymethylcytosine and nucleosome positioning. Finally, we have integrated chromatin state maps with our high depth nucleosome maps, to demonstrate the relationship of nucleosome positioning within a certain chromatin state. This has allowed us to determine the occupancy changes of nucleosomes within each state, the fuzziness of their binding, and the distance within the genome from one nucleosome to the other.

W-1057

IDENTIFICATION OF ADULT HUMAN SOMATIC CELLS THAT CAN GENERATE ALL THREE TISSUE LINEAGES

Thea D. Tlsty, Somdutta Roy
Pathology Department and UCSF Helen Diller Comprehensive Cancer Center, University of California San Francisco Pathology, San Francisco, CA, United States

The cellular mechanisms that generate heterogeneity and the emergence of cell-cell interactions that underlies development and the formation of functional tissues of the body are still a major mystery. Stress disrupts the status quo of tissue homeostasis and sets in motion cellular processes that result in dynamic cellular interactions to accomplish wound healing. Recent work from our group has identified multiple consequences of this disruption that range from changes in cell fate to modulation of the environmental niche. Under certain circumstances, restoration of homeostasis and repair is achieved. Under other circumstances, these consequences collude to create a pro-tumorigenic niche.
Surprisingly, the abovementioned stress-induced cellular processes can also activate the ultimate program of heterogeneity, pluripotency. We have identified cells within human adult tissue that can acquire a pluripotent state. These rare cells can be directly isolated from the human body and be shown to create functional derivatives of heart, brain, bone, cartilage, gut and many more tissues. This rare subpopulation is poised to transcribe pluripotency markers, Oct3/4, Sox2 and Nanog at levels similar to those measured in human embryonic stem cells and to acquire a pluripotent state sensitive to environmental programming. In vitro, in vivo and teratoma assays demonstrated that either a directly-sorted (uncultured) or a single cell (clonogenic) cell population from primary human tissue has the ability to differentiate into functional derivatives of each germ layer, ectodermal, endodermal and mesodermal. In contrast to other cells that express Oct3/4, Sox2 and Nanog, these human endogenous Plastic Somatic cells (ePS cells) are mortal, express low telomerase activity, expand for an extensive but finite number of population doublings, and maintain a diploid karyotype before arresting in G1. These cells may provide insights into the origins of tissue (and tumor) heterogeneity and the dynamic cellular interactions that underlie tissue generation.

W-1058

ANALYSIS OF PROTEIN-PROTEIN INTERACTIONS USING BAC-TRANSGENIC HUMAN PLURIPOTENT STEM CELLS AND THEIR NEURONAL PROGENY

Jonas Doerr[1], Marco Y. Hein[2], Ina Poser[3], Irina Solovei[4], Jerome Mertens[1], Heinrich Leonhardt[5], Anthony A. Hyman[3], Matthias Mann[2], Philipp Koch[1], Oliver Brüstle[1]
[1]Inst of Reconstructive Neurobiology, Bonn, Germany, [2]Max Planck Institute of Biochemistry, Martinsried, Germany, [3]Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany, [4]Biozentrum, Department of Biology II Anthropology & Human Genetics, Munich, Germany, [5]Department of Biology II Anthropology & Human Genetics, Munich, Germany

While contemporary approaches in cell-based disease modeling have been focusing mostly on the effects of defined mutations on the cellular phenotype, the assessment of underlying alterations in the interactomes of disease-relevant proteins has faced several technical challenges. First, experiments were typically conducted using

overexpression paradigms resulting in unphysiologically high protein levels and thus unspecific interactions. Second, such studies have been relying mostly on transformed cell lines, which enable mass production of cells but do not represent a tissue-specific proteomic environment. We aimed at addressing these issues by BAC-based expression of tagged proteins in pluripotent stem cell-derived long-term self-renewing neuroepithelial stem cells (lt-NES cells), a stable and robust cell population, which generates authentic human neurons with high fidelity. Tagged proteins were found to be expressed at endogenous levels, and FISH analyses revealed an average integration rate of one copy per genome. Correct size and compartmentalization of the tagged proteins could be confirmed by high-resolution live cell imaging and Western blot analysis. Employing this approach, we generated multiple cell lines, which harbor tagged proteins exhibiting altered expression in human developmental disorders, cancer and neurodegeneration, including PCNA, AURKA, CDK2AP1, RUVBL2, the Methyl CpG Binding Protein 2 (MECP2) and the Alzheimer's disease-associated proteins Nicastrin (NCSTN) and Valosin-containing protein (VCP). Using a label-free, quantitative affinity purification-mass spectrometry approach, we identified numerous novel interaction partners of these proteins. Direct comparison of the interactomes of proliferating lt-NES cells and their neuronal progeny further revealed changes in the composition of several chromatin remodeling complexes, suggesting that our system suffices to identify developmental switches in such complexes.

Pancreatic Cells

W-1063

STEM CELL BASED MODELS OF MONOGENETIC FORMS OF DIABETES

Dieter Egli[1], Linshan Shang[1], Haiqing Hua[1], Rudolph Leibel[2]
[1]NYSCF, New York Stem Cell Foundation, New York, NY, United States, [2]Pediatrics, Columbia University, New York, NY, United States

All forms of diabetes are caused by an inability of beta cells in the pancreas to provide sufficient insulin in response to elevated blood glucose levels. Stem cell models of diabetes could be useful to study the mechanisms of beta cell failure, and to investigate how beta cell function can be restored. To determine whether stem cell derived beta cells faithfully reflect the phenotype of a diabetic subject, we generated induced pluripotent stem cells from diabetics carrying mutations in genes involved in beta cell function, including glucokinase (GCK) and wolframin (WFS1). We found that beta cells derived from these stem cells reflect key aspects of the phenotype of the diabetic subject: GCK mutant stem cell lines show a reduced ability to secrete insulin in response to glucose, while WFS1 mutant cells are highly sensitive to endoplasmatic reticulum stress. Importantly, these phenotypes can be reverted upon gene sequence correction by homologous recombination. Our disease models provide an entry point into studying the effect of specific genotypes on beta cell function.

W-1064

ACTIVATION OF ADULT PANCREATIC STEM CELLS IN TYPE 1 DIABETES AND THEIR CONTRIBUTION TO β-CELL REGENERATION

Rozita Razavi, Sarah Abdullah, Derek van der Kooy
Univ of Toronto, Toronto, ON, Canada

The contribution of pancreatic stem cells in β-cell regeneration of adult tissue has been shown in pancreatic injury models. However, these studies have suggested the de-differentiation or trans-differentiation of adult pancreatic cells such as ductal and acinar cells as the main source of β-cell neogenesis.
Here we show the presence and contribution of a rare pancreatic (islet) cell population under diabetic conditions that contributes to β-cell regeneration in the adult tissue. We had previously shown that these pancreatic multipotent stem cells (PSCs) exist in the adult tissue and are capable of producing pancreatic and neuronal progenies in-vitro as well as in-vivo. These cells express insulin but lack the expression of β-cell specific glucose transporter, Glut2, or express it at very minimal levels.

We first used Streptozocin (STZ) that enters β-cells through Glut2 and destroys them, to induce diabetes in mice. Using colonal sphere assay, we observed that the STZ-induced diabetic islets have greater numbers of PSCs that also produce larger spheres. Moreover, the STZ-induced diabetic spheres produce more insulin+ progenies when differentiated.
In order to test the effects of β-cell stress on PSC function, we used NOD and NOD.Scid mice that show abnormal β-cell hyperactivity at around weaning time. At this time the autoimmune reaction starts in NOD mice, which eventually leads to β-cell loss and diabetes. In NOD.Scid, due to lack of immune response, only β-cell hyperactivity but no major physiological complications such as diabetes occurs. We observed that in NOD mice the number of PSCs significantly declines around 6 weeks of age. Since insulin is a major target for autoimmune reaction in Type1 diabetes (T1D) in NOD, and because PSCs also express insulin, this observation is expected. However, in the absence of autoreactivity and presence of β-cell stress, in NOD.Scid, similar to STZ model, the number and the size of PSC colonies increase. NOD snd NOD.Scid PSC colonies also produce significantly higher numbers of insulin+ progenies compare to the proper control strain, NOR mice.Interestingly we observed similar findings in human diabetic samples when compared with PSCs obtained from healthy donors' tissue.
To study the involvement of PSCs in in-vivo β-cell regeneration during diabetes, we used a doxycycline (dox) induced- β-cell -specific diphtheria toxin expressing transgenic mouse model. These mice develop diabetes after 7 to 10 days of dox treatment. However, when the dox treatment ends, β-cell regeneration rescues normoglycemia in these animals. Previous studies suggested β-cell self replication as the mechanism of regeneration in this diabetes model. Although in these studies, insulin was used only as a mature β-cell marker, thus the possible contribution of PSCs was ignored.
As was expected, at the onset of diabetes, the number of PSCs is significantly reduced in dox treated mice. However, throughout the recovery, there is a significant increase in the number and size of PSC spheres that declines and reaches the control level after 8 weeks. The number of insulin producing progenies from the PSC colonies also follows the same pattern in these mice.
Collectively, our findings suggest that human and mouse diabetic PSCs are more proliferative and potent in producing insulin+ cells. Moreover, when conditions allow, the adult pancreatic stem cells' contribution to β-cell regeneration can reverse diabetes.

W-1065

HUMAN PANCREATIC PROGENITOR CELLS IN 3D ORGANOID CULTURE AND GENERATION OF INSULIN-PRODUCING CELLS

Nerys A. Williams[1], Cindy JM Loomans[1], Léon CA van Gurp[1], Femke CAS Ringnalda[1], Toshiro Sato[2], Marten A. Engelse[3], Ton J. Rabelink[3], Hans Clevers[1], Eelco JP de Koning[1]
[1]Hubrecht Institute, Utrecht, Netherlands, [2]Department of Gastroenterology, School of Medicine, Keio University, Tokyo, Japan, [3]Department of Nephrology/Clinical Islet Transplantation, Leiden University Medical centre, Leiden, Netherlands

An attractive regenerative medicine approach towards β-cell replacement therapy is the derivation of insulin-producing cells from adult stem/progenitor cells. In the embryo, pancreatic multipotent progenitor cells (MPCs) give rise to all cell types of the pancreas. Identification of putative human pancreatic MPCs and insight into their plasticity can provide important clues for generation of insulin-producing cells.
Human islet-depleted pancreatic tissue was cultured using a novel 3-dimensional (3D) Matrigel-based system. With this system, pancreatic tissue could be expanded into complex 3D structures, henceforth referred to as 'organoids. Endocrine differentiation capacity of these organoids was analysed by transplantation of the organoids under the kidney capsule of immunodeficient mice. Putative progenitors were isolated by flowcytometry using the Aldefluor kit to detect cells with high Aldh protein expression. Differential gene expression profiles of sorted $Aldh^{hi}$ vs. $Aldh^{lo}$ fractions were obtained by microarray, and results were validated by qPCR and IHC. The colony-forming capacity of $Aldh^{hi}$ and $Aldh^{lo}$ cells was determined by plating 2-200 cells/µl Matrigel.

Organoids expanded for 7 days' culture resemble the embryonic ductal tree, with budding structures including tip-trunk sections. Expansion can be achieved over several passages for up to 3 months. Immunohistochemical analysis shows that expanded organoids are predominantly ductal in origin (92% $Ck19^+$), with expression of pancreatic progenitors markers ($Pdx1^+$, $Sox9^+$) and pronounced proliferative activity ($Ki67^+$) in the tip section of budding structures. Organoids transplanted under the kidney capsule of immunodeficient mice, give rise to insulin-positive cells (<1% of transplanted tissue), demonstrating that organoids have endocrine differentiation capacity. Aldefluor staining of expanded organoids *in vitro* reveal a high expression of Aldh exclusively in tip cells of budding structures. After cell sorting, clonogenic assays show 3% of $Aldh^{hi}$ single cells initiate organoid growth, whereas $Aldh^{lo}$ cells do not. Expression of genes previously described as multipotent markers of tip cells of the branching ductal tree during embryonic development (*Pdx1*, *Ptf1a*, *Myc* and *Cpa1)* is 3- to 7-fold higher in $Aldh^{hi}$ cells compared to $Aldh^{lo}$ cells. At the protein level, Cpa1 is co-expressed with Aldh in tips, and these cells are negative for the mature acinar marker amylase.
Our data indicate that 3D culture of human pancreatic tissue derived from the ductal compartment gives rise to pancreatic organoids that harbour progenitor cells at the branching tips resembling pancreatic development. Taken together with the generation of insulin-positive cells from organoids after transplantation *in vivo*, these data illustrate the potential of human postnatal pancreatic cells derived from the ductal compartment as an alternative cell source for β-cell therapy.

W-1066

CHEMICALLY TRANSDIFFERENTIATED FIBROBLAST FROM DIABETIC PATIENTS GENERATES PANCREATIC ENDODERM LIKE-CELLS IN VITRO AND GLUCOSE-RESPONSIVE INSULIN-SECRETING CELLS IN VIVO

Federico Pereyra-Bonnet, Maria Laura Gimeno, Nelson Argumedo, Johana Cardozo, Sung Ho Hyon, Marcelo Ielpi, Emiliana Herrero, Carla Gimenez, Marta Balzaretti, Patricia Fainstein-Day, Monica Loresi, Leon Litwak, Pablo Argibay
ICBME, Hospital Italiano, Buenos Aires, Argentina

Transdifferentiation of skin fibroblasts is a promising alternative for autologous replacement of pancreatic cells in patients with type 1 diabetes (T1D). However, the existing transdifferentiation technique uses genetic modifications which encompass clinical risks. We report here the transdifferentiation of skin fibroblasts from T1D patients into pancreatic like cells using only drug-based induction. For this goal, skin fibroblasts were extracted from two patients with T1D and one healthy volunteer and were cultured *in vitro* following a sequential multistep differentiation protocol including IGF1, Nicotinamide, Exe-4, ITS, B27 and glucose during 30 d. The chemically transdifferentiated cells showed islet-like cluster morphology and expressed the key genes and pancreatic transcription factor (e.g. INS, GCG, STT, PAX4, NGN3 and PDX1) detected by RT-PCR. We observed that our chemical protocol provoked modifications on a transcriptome-wide scale by microarrays. Between 655 and 1060 genes were either up-regulated or down-regulated by more than 2-fold in transdifferentiated cells compared to untreated cells. Some of the up-regulated genes were directly related to the pancreatic lineage or related with chromatin remodeling as INSIG1, NKX2.2, LOC651872, TGM2, TGF-b ligands, Nestin, BMPs and Smads, but none of them matched the typical markers hESC demonstrating the transdifferentiation nature of our protocol. Some of the down-regulated genes were related to the fibroblasts, such as CD34, Elastin, Filamin B, COL12A1 and COL8A1. The qPCR analysis showed that in transdifferentiated cells GCG and INS were up-regulated, and ASPN and MEOX2, two genes related to fibroblasts, were both down-regulated by more than 10-fold. PDX1 promoters were hypomethylated whereas OCT-4 and NANOG were isomethylated, in comparison with parental fibroblasts. The immunocytochemical and Inmunolite ® analysis detected Glucagon (in 30% of the cells) and C-peptide in *in vitro* transdifferentiated cells. Yet transdifferentiated *in vitro* cells showed pancreatic gene expression and proteins, these cells did not respond to glucose stimuli, resembling an inmature pancreatic endoderm stage. In *in vivo* trials, NUDE mice were transplanted intrapancreatically with $3\text{-}9x10^5$ transdifferentiated cells (n=6) or PBS (Sham; n=4) and non-transdifferentiated fibroblasts as a negative control (n=4). Fifteen days later the mice were treated with streptozotocin (STZ). Mice with implanted transdifferentiated cells (50%; n=3) showed detectable levels of human insulin (pM<200) and no teratoma formation. Sham and 30 d fibroblast control mice did not show detectable serum levels of human insulin. Moreover, one

of the 4 mice challenged showed a human insulin-secreting glucose-responding behavior (4-fold increase), demonstrating functionality of transdifferentiated cells *in vivo*. Additionally, transdifferentiated cells prevented the mice from high glycemia peaks ($p<0.006$) and weight loss above 10-15%, even though diabetes was not reversed during the 15 day monitoring phase post STZ. These findings demonstrated that using only drug-based induction could transdifferentiated fibroblasts from T1D patients, resulting in pancreatic endoderm like-cells *in vitro* and responsive insulin-secreting cells *in vivo*. This novel technique opens new possibilities for "transgenic-free" regenerative medicine in patients with T1D and demonstrated the potential plasticity in the adult non-stem cells.

W-1067

A STEM CELL MODEL OF DIABETES DUE TO GLUCOKINASE DEFICIENCY

Haiqing Hua[1], Linshan Shang[2], Hector Martinez[2], Matthew Freeby[1], Thomas Ludwig[1], Liyong Deng[1], Ellen Greenberg[1], Charles LeDuc[1], Wendy Chung[1], Robin Goland[1], Rudolph Leibel[1], Dieter Egli[2]
[1]Columbia University, New York, NY, United States, [2]New York Stem Cell Foundation, New York, NY, United States

Loss of beta cell mass and/or beta cell function, leading to deficiency of insulin relative to metabolic need and causes diabetes. To determine whether stem cell-derived beta cells recapitulate the cell-autonomous molecular/cellular phenotypes of endogenous beta cells of patients with a monogenic form of diabetes, we generated induced pluripotent stem cells (iPSCs) from subjects with maturity onset diabetes of the young (MODY). We have differentiated these stem cells *in vitro* into beta-like cells and have also transplanted pancreatic endoderm into immunocompromised mice to promote the differentiation of these cells into functioning beta cells. Using homologous recombination-mediated gene targeting, we were able to correct the mutated gene sequence. We found that these stem cell-derived beta-like cells accurately reflected beta cell phenotypes of MODY subjects, and that these phenotypes were fully reverted upon gene correction. These cells can be used for mechanistic analyses of these and genetic causes of diabetes.

W-1068

THE GCTM-5 EPITOPE ASSOCIATED WITH THE THE MUCIN-LIKE GLYCOPROTEIN FCGBP MARKS DUCTAL PROGENITOR CELLS IN LIVER, ESOPHAGUS, AND PANCREAS

Alison Farley[1], David Braxton[2], Kouichi Hasegawa[3], Martin F. Pera[1]
[1]Univ of Melbourne, Melbourne, Australia, [2]Emory University, Atlanta, GA, United States, [3]Kyoto University, Kyoto, Japan

Previously we described a monoclonal antibody GCTM-5 that is reactive with a large glycoprotein complex present on the surface of stem and progenitor cells of endodermal origin. There are a limited number of specific cell surface markers for endodermal progenitor cells. Therefore, in this study we sought to characterize further the expression of the GCTM-5 epitope in normal and diseased tissues. The antigen was strongly expressed on hepatoblasts at early stages (7.5 weeks) of human liver development. Later in gestation, the marker was found on periportal hepatocytes and the ductal plate, a structure that contains a bipotential stem cell population that persists in the Canal of Hering in the adult liver. Cells with the features of stem or progenitor cells in the ducts of the normal adult liver, pancreas, and esophagus all stained strongly, as did precancerous cells in the esophagus (intestinal metaplasia) and pancreas (pancreatic intraepithelial neoplasia). Submucosal gland cells of the esophagus, now regarded as a putative precursor of intestinal neoplasia, were also reactive with GCTM-5. Five out of seven cultured cell lines from pancreatic ductal carcinoma tested showed cell surface staining by indirect immunofluorescence and flow cytometry. The antigen secreted or shed into the culture medium by the antigen positive pancreatic carcinoma cell lines was not well resolved in immunoblots of conventional SDS-polyacrylamide gels, but migrated as a single diffuse band with an apparent MR of ~850 kDa in native Coomassie polyacrylamide gel electrophoresis. The large mucin-like glycoprotein FCGBP represented a major component of this multiprotein complex. Immunohistochemistry on tissue microarrays showed strong cell surface staining on 100/116 cases of pancreatic ductal carcinoma, but the antigen

was absent from most cases of colorectal, ovarian, breast or lung cancer. The GCTM-5 antigen identifies progenitor cells in ducts within tissues of endodermal origin that are implicated in repair and neoplasia.

W-1071

PDX1 CONTROLS A GENE REGULATORY NETWORK REQUIRED FOR ESTABLISHING HUMAN PANCREATIC ENDODERM IDENTITY

Ruiyu Xie[1], Allen Wang[1], Tommy C. Harper[1], Logan J. Everett[2], Hee-Woong Lim[2], Olivia G. Kelly[3], Allan J. Robins[3], Kyoung-Jae Won[2], Klaus H. Kaestner[2], Maike Sander[1]

[1]Department of Pediatrics and Cellular & Molecular Medicine, University of California San Diego, La Jolla, CA, United States, [2]Department of Genetics and Institute for Diabetes, Obesity and Metabolism, University of Pennsylvania, Philadelphia, PA, United States, [3]ViaCyte Inc., San Diego, CA, United States

A major goal in efforts to establish a curative diabetes therapy is to generate transplantable replacement pancreatic beta-cells from patient-derived pluripotent cells. The first step in producing cells of the pancreatic endocrine lineage from pluripotent stem cells is to establish pancreatic identity in vitro. Using a pancreatic differentiation protocol of human embryonic stem cells (hESCs) that recapitulates embryonic progression through multiple lineage intermediates, we generated genome-wide mRNA expression profiles by RNA-seq and demonstrated that the establishment of the pancreatic endocrine lineage involves stepwise activation of pancreas-specific genes. Here, we show that induction of the earliest pancreatic factor PDX1 at the primitive foregut stage is necessary to initiate and promote pancreatic lineage progression. Knockdown of PDX1 with a specific shRNA triggers distinct changes in gene expression including loss of pancreatic progenitor genes SOX9, NKX6.1, and PTF1A. Thus, much like in mouse development, PDX1 is required for the developmental progression of human pancreatic endoderm. In addition, genome-wide multistage studies by ChIP-seq reveal that induction of pancreatic endoderm is accompanied by progressive removal of H3K27me3 from a cluster of pancreatic endoderm genes, such as SOX9, NKX6.1 and PTF1A, as cells transition from the primitive foregut stage to a more differentiated state. Since induction of the pancreatic program coincides with the loss of H3K27me3, we suggest that PDX1 may be involved in the activation of a pancreatic-specific gene network by assisting in the establishment of an “active” chromatin landscape. This may be facilitated by the recruitment of histone demethylases, which could remove the repressive H3K27me3 chromatin mark. We have, thus, established a system that enables us to gain a more mechanistic understanding of pancreatic lineage specification by identifying interactions between transcription factors and histone modifiers during this dynamic process.

W-1072

IN VITRO REPROGRAMMING OF ADULT PANCREATIC DUCT CELLS TO INSULIN-PRODUCING BETA CELLS BY DEFINED TRANSCRIPTION FACTORS

Takatsugu Yamada[1], Claudia Cavelti-Weder[1], Lili Guo[1], Arun Sharma[1], Weida Li[2], Qiao Zhou[2], Susan Bonner-Weir[1], Gordon C. Weir[1]

[1]Islet Cell and Regenerative Biology, Joslin Diabetes Center, Boston, MA, United States, [2]Stem Cell and Regenerative Biology, Harvard Stem Cell Institute, Cambridge, MA, United States

Background: The reprogramming success of iPS cells opened the possibility of converting one mature cell type directly into another by forced expression of transgenes. We have previously shown that injection of adenoviral vectors encoding three pancreatic transcription factors, *Pdx1*, *Ngn3* and *Mafa*, into mouse pancreases results in reprogramming of exocrine cells to insulin-producing cells in sufficient numbers to reverse diabetes. **Aim**: We hypothesized that the cultured pancreatic duct cells (PDCs) could be reprogrammed to become insulin-producing beta cells by forced expression of transgenes. **Methods**: PDCs were isolated from adult MIP-GFP transgenic mice, allowing insulin-expressing cells to be detected by GFP fluorescence. The cultured PDCs were transfected by an adenoviral vector carrying a polycistronic construct Ngn3/Pdx1/Mafa/mCherry (Ad-M3C) or mCherry sequence alone (Ad-C) as

a control. In addition, we examined the effect of GLP-1 receptor agonist, exendin-4 on the reprogramming. **Results**: 8-12% of cultured PDCs were reprogrammed to GFP-positive insulin-expressing cells by transduction of Ad-M3C. Administration of Ad-M3C resulted in increased expression of beta cell markers insulin 1 and insulin 2, and exendin-4 enhanced this expression. Expression of other beta cell markers, neuroD and GLP1R, were also significantly upregulated in the reprogrammed cells. The total amount of insulin release into the media and insulin content in the cells were significantly higher in the cells transduced by Ad-M3C than those by Ad-C. Furthermore, exendin-4 had a marked effect not only on the insulin content but also on the insulin secretion of the reprogrammed cells. Ad-M3C-transduced cells did not secrete insulin in response to increased glucose, suggesting incomplete differentiation to beta cells. **Summary**: Cultured PDCs have been reprogrammed into insulin-producing beta cells by adenoviral delivery of three pancreatic transcription factors (Ngn3/Pdx1/MafA). Exendin-4 could enhance this process. This is the first report describing *in vitro* direct conversion of cultured mature PDCs to generate new insulin-producing cells. This study provides novel insights into the reprogramming of cultured PDCs to insulin-producing beta cells as the most promissing approach for beta cell replacement therapy for diabetes.

W-1073

DEVELOPMENT OF AN EFFICIENT DIFFERENTIATION METHOD FROM IPSCS/ESCS INTO PANCREATIC EXOCRINE LINEAGES TOWARDS NOVEL PANCREATIC DISEASE MODELS

Michinori Funato[1], Taro Toyoda[1], Yasushi Kondo[1], Yoshiya Hosokawa[1], Tomomi Sudo[1], Keisuke Okita[1], Isao Asaka[1], Motonari Uesugi[2], Zen-Ichiro Kato[3], Akira Ohta[1], Shinya Yamanaka[1], Naomi Kondo[3], Kenji Osafune[1]
[1]Center for iPS Cell Research and Application (CiRA), Kyoto University, kyoto, Japan, [2]Institute for Chemical Research, Kyoto University, Uji, Japan, [3]Department of Pediatrics, Graduate School of Medicine, Gifu University, Gifu, Japan

Human induced pluripotent stem cells (iPSCs), which take over individual patient characteristics, have the potential to be used in the study of complex diseases that result from not only genetic but also a mixture of genetic and environmental factors. Therefore, the work on establishment of directed differentiation methods from iPSCs/embryonic stem cells (ESCs) into a variety of somatic cell types has been vigorously performed around the world. The generation of pancreatic exocrine cells will be required for studying the disease mechanisms of acute pancreatitis, chronic pancreatitis, and congenital pancreatic exocrine dysfunction including Shwachman-Diamond syndrome (SDS) and Pearson syndrome. Although some previous reports have described that pancreatic exocrine cells can be differentiated from mouse and human iPSCs/ESCs, the efficient differentiation methods for the cell types have not been established so far. Herein, we report the development of an efficient differentiation protocol from iPSCs/ESCs into pancreatic exocrine lineages. We have slightly modified a previously published protocol from human iPSCs/ESCs into pancreatic progenitor cells, and obtained the highly efficient induction (approximately 70% to 80%) of PDX1 (+) pancreatic progenitor cells that could give rise to all pancreatic cell types including exocrine cells. Then, high-throughput screening (HTS) of chemical compounds was performed, and we identified a small molecule that can efficiently induce pancreatic progenitor cells into AMYLASE (+) cells at approximately 30% to 40% of induction efficiency. The small molecule proved to act synergistically with indolactam V (a protein kinase C activator), resulting in the generation of approximately 50% to 60% of total cells expressing AMYLASE. These AMYLASE (+) cells induced with the chemical compound expressed other pancreatic exocrine lineage markers, such as CPA1, LIPASE and ELASTASE, and showed the synthesis and release into the culture medium of functional alpha-AMYLASE protein. In contrast, the differentiation into pancreatic ductal cells or endocrine lineages was not significantly induced by the small molecule treatment. Furthermore, we found that the chemical compound also induced pancreatic exocrine lineages from mouse ESCs-derived pancreatic progenitor cells. These results suggest that our differentiation protocol with a novel chemical compound can efficiently produce pancreatic exocrine cells from mouse and human iPSCs/ESCs, and that the exocrine cells may be used for elucidating the mechanisms of pancreatic development as well as for creating novel in vitro disease models.

W-1074

AN ARTIFICIAL EXTRACELLULAR MATRIX PROTEIN SUPPORTS DIFFERENTIATION OF COLONY-FORMING CELLS FROM POSTNATAL MOUSE PANCREAS INTO BETA-LIKE CELLS IN VITRO

Nadiah Ghazalli[1], Alborz Mahdavi[2], Tao Feng[1], Liang Jin[1], Jasper Hsu[1], David A. Tirrell[2], Arthur D. Riggs[1], Hsun Teresa Ku[1]

[1]*Diabetes & Metabolic Diseases Research, Beckman Research Institute of City of Hope, Duarte, CA, United States,* [2]*Department of Bioengineering, California Institute of Technology, Pasadena, CA, United States*

Pancreatic stem and progenitor cells are potential sources for producing unlimited insulin-expressing beta cells to treat type 1 diabetes. In our previous studies, we established two pancreatic colony assays; one contains Matrigel, and the other an artificial extracellular matrix (ECM) protein containing a laminin-IKVAV sequence (termed laminin hydrogel). These colony assays allow quantitative and functional assessment of colony-forming cells isolated from adult murine pancreas, which we found to contain cells capable of self-renewal and differentiation into beta-like cells in vitro. The purpose of the current study is to determine whether postnatal pancreas also contains colony-forming cells, and whether the laminin-IKVAV sequence in the laminin hydrogel is necessary for their biological functions. Postnatal pancreas (1 week old) was dissociated into single cell suspension by collagenase B and DNase I and plated into methylcellulose semisolid media containing either 1) Matrigel, 2) laminin hydrogel, or 3) a protein hydrogel with a control scrambled sequence. We found that one week after plating, approximately 2.5, 1.3 or 1.5% of plated cells gave rise to colonies in the presence of laminin hydrogel, scrambled hydrogel or Matrigel, respectively. Cells supported by both types of hydrogel proteins expressed higher levels of endocrine and acinar markers, whereas those supported by Matrigel had higher ductal gene expression. Laminin hydrogel further enhanced expression of insulin genes compared to the scramble control. Cultures supported by laminin hydrogel, scramble control or Matrigel contained approximately 12, 6 or 1% C-peptide+ (a surrogate marker for insulin) cells, respectively, as determined by flow cytometry analysis. Individually handpicked colonies (n=6 from each group) developed from cultures containing laminin hydrogel but not other ECM proteins expressed higher level of Insulin2 gene by microfluidic qRT-PCR analysis. These colonies were C-Peptide positive as determined by whole-mount immunostaining. When challenged with high concentrations of D-glucose, laminin hydrogel-supported colonies were able to secrete C-Peptide into culture media. In summary, these results demonstrate that laminin hydrogel and Matrigel have opposing effects on cell lineage differentiation, and laminin-IKVAV sequence is necessary to support the development of insulin-secreting beta-like cells in vitro. These results identify postnatal pancreas as a potential source of beta-cell progenitor cells that could further be explored for regenerative medicine.

W-1075

CLONAL HETEROGENEITY WITHIN THE EXOCRINE PANCREAS

Damian Wollny, Xiaokang Lun, Ana Martin-Villalba
German Cancer Research Center (DKFZ), Heidelberg, Germany

The pancreas consists of two functional units: the hormone producing endocrine system and the digestive enzyme producing exocrine pancreas. Much effort has been made to investigate if the endocrine system is organized hierarchically. However the progress made in the past decade concluded that the endocrine system is not organized in a hierarchical way and that all beta cells contribute equally to growth and maintenance. If this is also the case for the exocrine pancreas remains elusive. Here we use multicolor lineage tracing to study the clonal contribution of cells within the acinar cell population, the major compartment of the exocrine pancreas. While examining potential progenitor populations we find substantial differences in the proliferation dynamics of single clones among acinar cells. As a complementary in vitro approach to assess clonal heterogeneity, we examine the organoid-forming capacity of these cells. In this assay, we identified a unique subpopulation of acinar cells with the ability to give rise to organoids. Thus, although the acinar population, similarly to the beta cells among the endocrine cells, is often considered as a homogeneous population we find clonally heterogeneous contribution to growth and maintenance of pancreas homeostasis. Furthermore these data challenge the perception of the pancreas as a quiescent organ.

Liver Cells

W-1081

HUMAN PARTHENOGENETIC STEM CELL-DERIVED HEPATOCYTES CORRECT SERUM BILIRUBIN IN GUNN RATS

Larissa Agapova, Alina Ostrowska, Trudy Christiansen-Weber, Tiffany Chu, Ruslan Semechkin
ISCO, Carlsbad, CA, United States

Human liver cell transplantation trials are hampered by the shortage of donor tissue therefore different type of stem cells are being investigated for their potential use in regenerative therapy. Hepatocyte-like cells (HLC) derived from human parthenogenetic stem cells (hpSC) might constitute an easily available source to obtain the large numbers of transplantable cells to treat liver-based inborn errors of metabolism. Here we demonstrate that after the optimized endodermal differentiation in vitro hpSC reaggregate and grow into HLC that are developmentally immature hepatocytes, similar in expression profile to fetal liver cells. Differentiated HLC were characterized by immunostaining, quantitative RT-PCR, biochemical function (LC/MS, ELISA, bioluminescent assays) and in vivo analyses. Hepatocyte phenotype of the obtained cells, was evidenced by demonstrating the enzymatic activity of phase I drug metabolizing enzymes, glycogen storing and the presence of liver-specific proteins, such as albumin, alpha-fetoprotein, alpha 1-antitrypsin and coagulation factors. To evaluate engraftment potential and functional repopulation in vivo, we have treated juvenile Gunn rats (a well established model of Crigler-Najjar disease) by a single intrasplenic injection with 10x106 of HLC, pre-labeled with CFSE. A histological post-surgery examination revealed normal appearance of engrafted cells which continued to have hepatic features. Most importantly, we demonstrated a significant decrease and long-term stabilization of bilirubin levels in the serum of tested animals in comparison with sham-treated controls. In immunosuppressed animals receiving therapeutic doses of HLC, serum indirect bilirubin declined significantly throughout the 19th week observation period. Multiple engrafted cells were observed in the periportal regions of the liver lobules. Many fluorescent cells were also clustered in small groups predominantly around the central veins. Moreover, at four months following treatment no adverse safety signals were detected. The overall liver structure of all tested rats appeared undamaged and no inflammation, tumorigenicity, or apparent rejection signs were observed. In summary, we demonstrated substantial grafting of inoculated hepatocyte-like cells in the recipient Gunn rats within 4 months which was associated with significant and stable reductions of serum bilirubin levels. The data strongly indicate the potential of parthenote-derived hepatocytes as an easily available source to obtain a large number of transplantable cells for regenerative treatments of metabolic liver diseases including Crigler-Najjar syndrome.

W-1082

PROLIFERATION IS INDEPENDENT OF MATURATION DURING THE DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS TOWARDS HEPATOCYTES

Changjun Zeng, Benjamin Tschudy-Seney, Xiaocui Ma, Tia Hackett, Wei Gao, Ahmad Hasan, **Yuyou Duan**
Internal Medicine, University of California Davis Medical Center, Sacramento, CA, United States

Adult hepatocytes lack the capacity to proliferate in vitro; this hinders their use including hepatocyte-dependent drug discovery and development. Human embryonic stem cells (hESC) are a potential source to generate viable, functioning hepatocytes for clinical application and pharmaceutical use. In this study, we attempted to determine how hESC-derived hepatocytes (hDH) proliferate during differentiation and maturation. hESC lines, H9 and ESI017, were differentiated towards hepatocytes under our established differentiation protocol. hESC was induced to definitive endoderm (DE), then DE were further differentiated towards hepatocytes for 15 days under differentiation medium, and for an additional 21 days with maturation medium. The differentiated cells were counted every other day from day 2 after the initiation of differentiation. In addition, cDNA was generated after RNA was extracted from the counted cells, and supernatant was collected at all time points. Cell counting showed that the cell proliferation peak appeared at day 8 after starting differentiation for both H9 and ESI017 lines, and this proliferation peak was not concentration-dependent. The total cell numbers showed a slight decrease every other day after the medium

was changed for maturation. qPCR results showed that the highest expression of alpha fetoprotein (AFP, immature marker) was between days 12-14, then its expression continued to decrease to the end of the time course. The expression of albumin (ALB, more mature marker) increased continuously to the highest peak at day 30. ELISA results showed that AFP in the medium was highest at day 14 then continued to go down until day 30, and ALB in the medium was detected from day 12 and continued to go up until day 30. These results indicated that the cells were differentiated at day 14, and were more matureat day 30 under our differentiation conditions. We changed our differentiation conditions by employing differentiation medium for the entire time-course or by changing maturation medium from day 8 instead of day 15. Although qPCR results showed that gene expression patterns were changed, the cells did not show proliferation after day 8 of the changed conditions. Expression of Ki67 and PCNA decreased after the differentiation started from DE, with the relative expression levels of Ki67 and PCNA being only 20% as high at day 8 when compared to day 2. This suggested that the proliferation is independent of the differentiation and maturation of hESC towards hepatocytes. Importantly, even though the cells do not proliferate after day 8, this never affected their function at later time points. During the non-proliferation period (days 9 to 30), hDH produced 100 ug ALB in the medium as determined by ELISA, showed a functioning biotransformation system as determined by the cellular uptake and excretion of Indocyanine Green, developed full metabolic function as determined by the metabolic profiling of the drug bufurarol, and could engraft and proliferate in livers after transplantation into immunodeficient mice. The relationship between telomere length change and cell proliferation is under investigation. Thus, our results help enhance our understanding of stem cell derivatives and their use, and also play an important role in developing optimal cryopreservation timing for hESC-derived hepatocytes.

W-1083

HEPATIC DIFFERENTIATION OF SIMIAN IPSCS: A TOOL FOR CELL THERAPY APPLICATIONS

Clara Steichen[1], Soumeya Abed[2], Sébastien Corbineau[1], Stany Chrétien[2], Anne Weber[1], Philippe Leboulch[2], Leïla Maouche-Chrétien[2], Anne Dubart-Kupperschmitt[1]

[1]Inserm U972, Villejuif, France, [2]Inserm U962/CEA/iMETI, Fontenay aux Roses, France

Pluripotent stem cell based therapy carries great potential in the treatment of liver disease. Indeed, stem cell-derived hepatocyte transplantation represents an alternative to orthotopic liver transplantation, which is limited by the lack of donor and the difficulty to expand hepatocytes in vitro. However, before any clinical application, the safety, efficacy and feasibility of this therapeutic approach has to be established in large animal models, including nonhuman primates, who are physiologically and phylogenetically very close to human. The aim of this work is to demonstrate that autologous simian iPSCs differentiated into hepatocytes are capable of integrating into the liver parenchyma of non-human primates with a sufficient degree of chimerism and functionality. However, despite the fact that many protocols already exist describing the differentiation of human pluripotent stem cells into hepatocyte-like cells, they cannot be directly transferred to monkey ESCs or iPSCs and little is known about the hepatocyte differentiation potential of these cells. A few protocols are already described, but the fonctionallity of simian-hepatocytes like cells in vitro is poorly studied, and their engraftment has not been evaluated yet.
We first generated iPSC lines from primary fibroblasts and bone marrow mesenchymal stem cells of Macaca fascicularis (cynomolgus). We used RT-PCR, flow cytometry and immunofluorescence assays to confirm the expression of the pluripotent endogenous genes OCT3/4, NANOG, SOX2, KLF4, Myc and SSEA4. Intramuscular injection of cy-iPSCs into immunocompromized mice induced the development of teratomas containing tissues of the three germ layers, validating the pluripotency of the cy-iPSCs. The next step was to differentiate these cy-iPSCs into hepatic progenitor cells and hepatocytes and to characterize them in vitro for their phenotype markers and hepatic functions. We have set up chemically defined conditions devoid of serum, to efficiently differentiate cy-iPSCs into endodermal cells showing the expression of specific combination of markers such as SOX17, GATA4, HNF3β and CXCR4. These cells have been differentiated into hepatic specified endoderm showing the expression of liver specific markers such as AFP and HNF4α. We are currently defining conditions to further differentiate these cells into hepatic progenitors and more mature hepatocyte-like cells. Specific liver functions such as albumine secretion, glycogene storage and cytochrome P450 induction capacity will be evaluated.

Given the strong expertise of our surgeon team who already defined protocols to perform efficient hepatocyte engraftment into nonhuman primates, this work should offer a proof-of-concept of autologous stem cell derived hepatocyte transplantation into nonhuman primates and an accurate model to evaluate the therapeutic efficacy and safety of this approach in human patients.

W-1084

SOX9 POSITIVE CELLS OF THE BILE DUCTS- A PROMISING TARGET FOR MAKING BETA CELLS FOR CELL THERAPY OF TYPE1 DIABETES PATIENTS

Anannya Banga, Ying Yang, Lucas Greder, James Dutton, Jonathan MW Slack
Genetics Cell Biology and Development, University of Minnesota, Minneapolis, MN, United States

We have investigated the reprogramming towards a beta cell phenotype of various developmentally related cell types, using the gene combination Pdx1 + Ngn3 + MafA separated by 2A peptides in an adenoviral construct (Ad-PNM). We found that hepatocytes are refractory and cannot be permanently reprogramed towards a beta cell state. But we have shown for the first time that Ad-PNM can uniquely target a progenitor population in the liver and reprogram them into insulin secreting ducts, expressing various markers of beta cells. We have identified this progenitor population as SOX9-positive population of cells residing in the biliary tract, those of which can be reprogramed to insulin secreting ductal structures and can rescue diabetes in mice long term. SOX9 positive progenitor cells are known to serve as a common progenitor compartment for the intestine, pancreas and liver, thus being more developmentally related is believed to possess a more open chromatin at the beta cell genes and can be considered as a promising target for making the desired cells for cell therapy of Type1 Diabetes. Henceforth we have discovered the potential of developmentally related SOX9 positive cells of the bile ducts towards attaining a beta cell fate which opened up new interests to make future beta cells from this progenitor population, thereby overcoming the constraints of using ES or iPS cells. Isolation of the SOX9 positive cells from the bile ducts in the liver using SOX9CreERT2 mice showed that they are a distinct population of epithelial cells, expressing ECAD and SOX9 but not EpCAM, a marker for liver stem cells. These epithelial cells can be isolated in culture from the digested liver as green GFP labeled balls and can be infected with the beta cell transcription factors in vitro to attain a beta cell fate. These cells upon reprograming invitro are found to express an array of beta cell markers along with insulin secretion in response to glucose. Ductal progenitor cells in adult pancreatic ducts and its ability to make beta like cells has been described long time back but its presence in the liver will make it relatively easy to isolate those cells by a small liver biopsy. We henceforth believe that SOX9 positive cells of the bile ducts as novel alternative cell type for making beta cells and holds enormous possibilities for generating autologous human beta cells for transplantation therapy.

W-1085

VEGFR2/KDR IDENTIFIES A HUMAN MESENCHYMAL HEPATIC PROGENITOR AND INSTRUCTS EARLY LIVER DEVELOPMENT

Orit Goldman[1], marion Sourisseau[1], Noelle Dziedzic[1], Corneo Barbara[2], Sunita D'Souza[1], Matthew Evans[1], Valerie Gouon-Evans[1]
[1]mount sinai school of medicine, new york, NY, United States, [2]2Neural Stem Cell Institute, Rensselaer, NY, United States

The capacity of embryonic stem cells (ESC) to form hepatocyte-like cells (hepatic cells) in culture provides a unique model system for liver developmental studies and the generation of transplantable cells for cell therapies to overcome the shortage of liver donors. Using human ESC lines, we have established conditions for efficient endoderm development assessed by the co-expression of CXCR4 and cKit. Isolated day5 CXCR4+cKit+ endoderm cells were further cultured in hepatic conditions. In order to examine the emergence of potential endothelial cells in hepatic cultures, previously shown to support hepatic differentiation (Han et al., Stem Cells, 2011), expression for endothelial markers VEGFR2 (KDR) and PECAM-1 (CD31) were monitored. Based on CD31 and KDR expression, three populations emerged sequentially: first the KDR+CD31- (K+C-) pre-hepatic cells negative for AFP protein (~50% at day 12), then the KDR+CD31+ (K+C+) endothelial cells (~5% at day12), and lastly the KDR-CD31- (K-C-) hepatic cells expressing AFP protein (~50% at day12).
Gene expression analyses by qPCR showed that K-C- and K+C- populations expressed equal transcript levels of the ventral endoderm marker GATA4 and the epithelial hepatic marker CK18. However, levels of the endoderm and hepatic markers FOXA2, AFP and Albumin were higher in K-C- cells compared to those in K+C- cells, suggesting that K+C- cells progressed to an intermediate hepatic fate. Interestingly, levels for the mesenchymal markers Vimentin and Snail1 were always higher in K+C- cells compared to those in K-C- cells and inversely correlated with levels of the epithelial marker E-Cadherin, compatible with a more mesenchymal phenotype of K+C- cells.
To determine whether the pre-hepatic K+C- cells represent a progenitor for the hepatic K-C- cells, hepatic potential was examined in a three-dimensional aggregate culture that favors maturation. In aggregates, virtually all K+C- cells expressed AFP protein, defining the K+C- cells as hepatic progenitors for the K-C- hepatic cells. To demonstrate the functionality of the human K+C- progenitors following further hepatic specification and maturation, we exploited the well-established ability of the hepatitis C virus (HCV) to infect specifically hepatocytes. This functional study revealed that K+C- progenitors supported re-

latively weak HCV replication as expected, yet this improved (40-fold) after further hepatic specification and maturation, to levels comparable to the K-C- hepatic cells and even the best-case Huh-7.5 hepatoma cell line.
Co-culture assays between the K-C- and K+C- populations demonstrated that K+C- cells constitute not only a pool of hepatic progenitors, but also a supportive niche in promoting hepatic specification and maturation of the K-C- hepatic cells. Blocking KDR function or an antibody or small molecules dramatically reduced Albumin transcript levels in K-C- hepatic cells. Induction of hepatic maturation (Albumin expression) was mostly mediated in a non-cell autonomous manner through KDR signaling expressed on the K+C- progenitors.
This study reveals that VEGFR2/KDR, previously thought to be restricted to mesodermal derivatives, is actually a marker for a mesenchymal endoderm-derived hepatic progenitor, and is also a functional receptor instructing early human liver development.

W-1086

AN IN VIVO LINEAGE TRACING STUDY IDENTIFIES AN UNEXPECTED FLK-1 EXPRESSING HEPATIC PROGENITOR

Songyan Han[1], Noelle Dziezdic[1], Alice Bourdon[1], Thomas Sato[2], Valerie Gouon-Evans[1]
[1]Developmental and Regenerative Biology, Mount Sinai School of Medicine, New York, NY, United States, [2]Developmental and Regenerative Biology, Nara Institute of Science and Technology, Nara, Japan

Complex cell-cell communication between endoderm and the microenvironment is required for proper liver development. FGF and BMP signaling from the cardiac mesoderm and the septum transversum, respectively, instructs hepatic specification of the adjacent ventral endoderm. Endothelial cells form a necklace around the specified hepatic endoderm and promote hepatoblast expansion. Using the mouse embryonic stem cell (ESC) differentiation system, we have recently demonstrated that endothelial cells are not only required for hepatic cell expansion, but are also required earlier for the hepatic specification of endoderm by dual repression of Wnt and Notch signaling [Han et al, Stem Cells, 2011].
In the attempt to track the emergence of endothelial cells in mouse ESC hepatic differentiation cultures, we examined expression for the early endothelial marker Flk-1 (VEGFR2, KDR) as well as the endoderm marker Foxa2. For this purpose, we used a reporter ESC line allowing us to isolate progenitors for endoderm based on brachyury and Foxa2 expression. Upon hepatic specification of isolated endoderm progenitors, two main populations consisting of a Foxa2+Flk-1- hepatic fraction and a Foxa2-Flk-1+ endothelial fraction developed. Surprisingly, a transient Foxa2+Flk1+ population also emerged and displayed bipotential hepatic and endothelial fates following further culture. This in vitro study suggested the existence of an unexpected endoderm-derived hepatic progenitor expressing the mesodermal marker Flk-1. Indeed, Flk-1 expression was thought to be restricted to mesodermal derivatives including endothelial, hematopoietic, cardiac and skeletal muscle precursors.
In order to provide an in vivo evidence for the existence of a Flk-1 expressing hepatic progenitor, mouse embryos from E8.0 (prior to hepatic specification), E9.5 (liver bud stage) and E13.5 (fetal livers) were examined for Flk-1 and the endoderm marker Foxa2 expression by immunohistochemistry. Scattered Flk-1+Foxa2+ cells were detected in endoderm and were strictly found in E8.0 embryos prior to hepatic specification. To demonstrate the hepatic fate of the Flk-1+Foxa2+ endoderm cells, an in vivo lineage tracing mouse model was analyzed in which all Flk-1 expressing cells as well as their progeny were labeled genetically. This mouse model expresses YFP under the ubiquitous Rosa26 locus specifically in Flk-1+ cells and progeny of Flk-1+ cells (Cre-Flk-1 x Rosa26-LoxpSTOPLoxpYFP). By tracking the YFP+ cells in developing fetal livers (E13.5), we determined that out of 36 fetal livers analyzed, all of them were consistently constituted with between 30% to 50% of YFP+ hepatoblasts that were derived from a Flk-1 progenitor. In turn, the YFP+ hepatoblasts gave rise to a subset of YFP+ adult hepatocytes based on albumin and HNF4α expression, as well as YFP+ cholangiocytes based on CK19 and Sox9 expression.
Altogether, this lineage tracing study provides for the first time the in vivo proof for the existence of an unexpected endoderm-derived hepatic progenitor expressing the mesodermal marker Flk-1.

W-1087

THE IDENTIFIATION OF CANCER STEM CELL MARKER IN HEPATITIS B VIRUS INFECTED PRIMARY HEPATOCYTE CELLS

YEN SHUN CHEN, KUAN WANG
Institute of Biological Chemistry, Taipei, Taiwan

The liver cirrhosis and hepatocellular carcinoma (HCC) disease is ranking as the third leading cause of cancer-related death and the fifth most common solid cancer in the worldwide. Several lines of evidence implicate HCC is correlated with the infection of hepatitis B virus (HBV) that activate and deregulate multiple signaling transduction pathways in hepatocarcinogenesis. The cancer stem cell (CSC) has been demonstrated in tumor progression, tumor resistance to chemo/radiation therapy and tumor relapse in HCC patients. It's important to elucidate the roles of putative target genes/proteins that should be taken into consideration when designing therapeutic strategies for HCC in the HBV infected liver. We investigated the signaling pathway of HNF-4alpha, EpCAM, CD13, $CD44^+$, $CD90^+$, CD133, Wnt/β-catenin, TGF-β, Notch and Hedgehog in the HBV infected live by the techniques of RT-PCR, flow cytometry and confocal microscopy. Here we provide a brief view of molecular signaling in CSCs related marker protein and present insights into new therapeutic strategies for targeting HBV-infected HCC.

W-1088

REGULATION OF CYTOCHROME P450 3A11 EXPRESSION IN MOUSE HEPATIC PROGENITOR CELLS BY BCL-6

Hiromi Chikada[1], Keiichi Ito[2], Ayaka Yanagida[2], Hiromitsu Nakauchi[2], Akihide Kamiya[1]
[1]*Laboratory of Stem Cell Therapy, Institute of Innovative of Science and Technology, Tokai University, Isehara, Japan,* [2]*Division of Stem Cell Therapy, Center for Stem Cell and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, Chiyoda-ku, Japan*

[Background]
Adult liver is a central organ for intermediary metabolism, in particular detoxification. Hepatocytes are major parenchymal cell type of the liver and express several metabolic genes. Many human hepatocytes are needed in regenerative medicine for severe liver diseases and analyses of drug metabolism. However, proliferation of mature hepatocytes *in vitro* is difficult in spite of their high regenerative capacity *in vivo*. Differentiation of highly proliferative hepatic progenitor cells to mature hepatocytes after expansion might provide a method to obtain large quantities of functional hepatic cells.
[Objective]
For effective maturation of hepatoblasts, fetal hepatic progenitor cells, into mature hepatocytes *in vitro*, we analyzed transcription factors regulating hepatic functional genes.
[Methods]
To search the candidate genes regulating hepatic differentiation, expression micro array analyses were performed in both adult hepatocytes and embryonic day 13 (E13) hepatoblasts. We considered genes whose expression levels were higher in adult hepatocytes than E13 hepatoblasts as the candidates. These genes were overexpressed using retovirus vectors in the culture system, which induced maturation of hepatoblasts by the addition of oncostatin M and extracellular matrices.
[Results]
We found several transcriptional and nuclear factors were highly expressed in mature hepatocytes but not E13 hepatoblasts. One of these candidate factors was B cell lymphoma (Bcl) 6. Overexpression of Bcl-6 increased expression of cytochrome P450 (CYP) 3A11, the important drug detoxification enzyme, in E13 hepatoblasts culture. In contrast, other detoxification enzymes (CYP2B10, CYP3A16, CYP7A1) and metabolic genes (tyrosine aminotransferase and carbamoylphosphate synthetase) were not up-regulated by overexpression of Bcl-6. Moreover, expression of transcriptional factors important in liver development such as hepatic nuclear factor 4α and 3β also weren't changed by overexpressed Bcl-6. These results suggested that Bcl-6 directly regulated expression of CYP3A11 in hepatic cells. Bcl-6 is a transcription factor consisting of C2H2 type Zinc finger and BTB/POZ domains. Therefore,

we analyzed the Bcl-6 effect on the CYP3A11 promoter using luciferase assay system. As a result, overexpression of Bcl-6 increased CYP3A11 reporter activity through the region from -1725 to -1523.
[Conclusions]
The present study suggests that Bcl-6 is a new regulator of mature hepatic function through the transcriptional regulation of CYP3A11.

W-1091

CHARACTERIZATION OF LIVER ORGANOID TISSUES COMPOSED OF MURINE HEPATIC PROGENITOR CELLS AND FIBROBLASTS IN DENSE COLLAGEN FIBRILS

Miho TAMAI[1], Hiroshi SAKAI[2], Shinichi Miyagawa[3], Eijiro ADACHI[4], Yoh-ichi TAGAWA[1]
[1]Graduate School of Bioscience and Biotechnology, Department of Biomolecular Engineering, Tokyo Institute of Technology, Yokohama-shi, Japan, [2]Shinshu University School of Medicine, Matsumoto-shi, Japan, [3]Shinshu University School of Medicine, Mastumoto-shi, Japan, [4]Kitasato University, Sagamihara-shi, Japan

Hepatic stem/progenitor cell is one of the promising cell-sources to restore liver mass and its functions. Although hepatic progenitor cells (HPC), such as oval cells, are induced by the administration of some hepatotoxins in experimental animals, such strategy should be inappropriate for the clinical setting. In recent, we succeeded to establish and long-culture HPC clones from non-parenchymal cell fraction of portal branch ligated lobes, PBL-HPCs, in mice. In this study, using the PBL-HPCs liver organoid tissues were tried to be reconstructed in an originally designed bioreactor system. The liver organoid tissue was investigated about transplantation into partially hepatectomized BALB/cA-nu/nu mice.
We succeeded to reconstruct the liver organoid tissues model using a HPCs, embryonic fibroblast, and collagen. The PBL-HPCs acquired the metabolic functions of detoxifying ammonium ions and urea synthesis in the presence of oncostatin M. Several liver-specific gene expressions, such as tryptophan 2,3-Dioxygenase were strongly detected in this system compared to that in the 2D-culture. We have found that angiogenesis was induced by transplantation of liver organoid tissues to the liver organoid tissues and surrounded tissue. A novel approach was demonstrated to generate transplantable liver organoid tissues with condensed collagen fibrils matrix. Based on the sufficient vascular support for the transplanted liver organoid tissues, we expect that it was established a local vascular network at the transplantation site would allow nutrient and gas transport to the grafted liver organoid tissues, and this would reduce graft loss. These results provide future avenues for the potential use of PBL-HPCs in the treatment of liver disease.

W-1092

LONG-TERM CULTURE OF HEPATOBLAST-LIKE CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Kazuo Takayama[1], Yasuhito Nagamoto[1], Kenji Kawabata[2], Katsuhisa Tashiro[2], Fuminori Sakurai[1], Masashi Tachibana[1], Hiroyuki Mizuguchi[1]
[1]Laboratory of Biochemistry and Molecular Biology, Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Japan, [2]Laboratory of Stem Cell Regulation, National Institute of Biomedical Innovation, Ibaraki, Japan

Hepatoblasts have ability to differentiate into both hepatic and biliary lineages, and to self-replicate. The establishment of self-renewing hepatoblast-like cells (HBC) from human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells would lead to a stable supply of the hepatocyte-like cells for medical application such as drug screening and liver cell transplantation. Although we have previously demonstrated that almost homogeneous HBC population could be generated from human ES/iPS cells (J Hepatol. 2012 Sep;57(3):628-36.), the functional characterization of human ES/iPS cell-derived HBC was still not enough. The human ES/iPS cell-derived HBC was maintained under the appropriate conditions. The expanded human ES/iPS cell-derived HBCs were epithelial cell adhesion molecule+, CD133+, alpha-1-fetoprotein+, albumin+, cytokeratin (CK) 7+, and CK19+. The human ES/iPS cell-derived HBC could differentiate into both the hepatocyte-like cells in presence of hepatocyte growth factor, oncost-

atin M, and dexamethasone, and the cholangiocyte-like cells in presence of epidermal growth factor and insulin-like growth factor 2. The human ES/iPS cell-derived HBC were maintained for more than 3 months. In addition, the human ES/iPS cell-derived HBC have potential to integrate into the liver parenchyma of immunodeficient mice that are damaged by carbon tetrachloride treatment. These results indicated that functional hepatoblasts could be generated from human ES/iPS cells, and they could be maintained in long-term culture. The expandable human ES/iPS cell-derived HBC might be manageable tools for drug screening, experimental platforms to elucidate mechanisms of hepatoblasts, cell source for human ES/iPS cell-based hepatic regenerative therapy.

W-1093

SEMI QUANTITATIVE ASSAY OF CYP3A4 EXPRESSION ALLOWS THE IDENTIFICATION AND SELECTION OF MATURE HUMAN STEM CELL DERIVED HEPATOCYTES

Ryan Rodriguez[1], Kristina Bonham[1], Shinichiro Ogawa[2], Carmina Blanco[1], Danajane Eymer-Vargas[1], Hai-Qing Xian[1], Gordon Keller[2], H. Ralph Snodgrass[1]

[1]VistaGen Therapeutics, Inc., South San Francisco, CA, United States, [2]McEwen Centre for Regenerative Medicine, Toronto, ON, Canada

One of the pitfalls of many pluripotent stem cell differentiation protocols is the extremely poor efficiency of producing mature hepatocytes. The cytochrome P450 3A (CYP3A) subfamily of enzymes is the most predominant in the human liver. In the fetal liver CYP3A7 is the predominant isoform, whereas there is an almost exclusive shift to the CYP3A4 isoform in the adult liver. Furthermore, CYP3A4 is responsible for the metabolism of half of the pharmaceuticals currently available, as well as playing a central role in steroid homeostasis. Most published stem cell differentiation reports demonstrate CPY3A7 expression, but little to no CYP3A4 expression or activity, indicating the lack of maturity of the hepatocyte-like cells being studied. In an effort to address this problem, we generated a human embryonic stem cell (hESC) reporter line containing a humanized version of the beta-lactamase reporter gene (hBLA) targeted to the 3' untranslated region of the CYP3A4 locus. We show the use of this cell line as a semi-quantitative tool for analyzing the expression of CYP3A4 in individual cells and for the selection of mature hepatocytes by fluorescence activated cell sorting. We demonstrate that this assay can be used to monitor the induction of CYP3A4, its expression level over time, and to measure experimental effects on the maturation of hepatocytes. Using an optimized protocol for the differentiation of hepatocyte-like cells, we demonstrate CYP3A4-hBLA expression in 25-40% of the unsorted differentiated cell population. Furthermore these cells show expected adult responses: 1) rifampicin induction, 2) metabolism of known CYP3A4 substrates, 3) albumin production and 4) phase II enzyme activities. Sorted CYP3A4-hBLA cells express levels of CYP3A4 mRNA approaching that in human adult liver on a per cell basis. These data suggest that these cells have many of the functional properties of mature hepatocytes. This system allows us to measure the expression of CYP3A4-hBLA on a per cell basis in response to experimental conditions, and treatments with drugs, and corroborate those data with cytochromes P450 enzyme activities, Phase II enzyme activities, and secretion of hepatic factors, such as albumin and urea. These multiple functional analyses provide a powerful system to evaluate the effects of test compounds on CYP3A4 expression and hepatocyte function, offering a valuable aid for assessing potential drug candidates for toxicity.

W-1094

COMPARATIVE ANALYSIS OF TRANSPLANTATION EFFICACY OF HUMAN IPS CELL-DERIVED HEPATIC CELLS AT VARIOUS DIFFERENTIATION STAGES IN MICE

Yasuhito Nagamoto[1], Kazuo Takayama[1], Katsuhisa Tashiro[2], Chise Tateno[3], Fuminori Sakurai[1], Masashi Tachibana[1], Kenji Kawabata[2], Kazuo Ikeda[4], Yasuhito Tanaka[5], Hiroyuki Mizuguch[1]

[1]Osaka university, Osaka, Japan, [2]National Institute of Biomedical Innovation, Osaka, Japan, [3]PhoenixBio Co. Ltd., Hiroshima, Japan, [4]Osaka City University, Osaka, Japan, [5]Nagoya City University, Nagoya, Japan

Human liver chimeric mice were typically produced by transplanting cryopreserved human hepatocytes into urokinase-type plasminogen activator-transgenic SCID mice (uPA/SCID mice), which are immunodeficient and undergo liver failure. Human hepatocytes transplanted into this model can repopulate the mice host liver, frequently replacing over 70% of the native liver with human hepatocytes. Recently, researchers have shown that these mice models are applicable for studies of hepatitis virus biology and human drug metabolism. However, human cryopreserved hepatocytes have disadvantages in the limit of their sources, costs and inability of genetic modification. Because hepatocyte-like cells differentiated from human induced pluripotent stem cells (iPS cells) have potential to resolve these problems, they are expected to apply for generation of human liver chimeric mice. We have established the efficient method for hepatic differentiation from human iPS cells by sequential transduction of forkhead box A2 (FOXA2) and hepatocyte nuclear factor 1 homeobox A (HNF1α) using adenovirus vector (J Hepatol. 2012 Sep;57(3):628-36.). The human iPS cell-derived hepatocyte-like cells expressed the similar gene expression levels of hepatocyte-related markers, such as *CAR*, *CYP3A4*, and *CYP2E1*, as compared with those of primary human hepatocytes. To generate human liver chimeric mice by transplanting the human iPS cell-derived hepatic cells, it is necessary to examine which cell type is the most suitable for cell transplantation. In particular, it has not been well-investigated whether human iPS cell-derived definitive endoderm (DE) cells, hepatoblasts, or hepatocyte-like cells will provide better results. To examine whether the transplanted human iPS cell-derived cells could integrate into the liver of uPA/SCID mice, *in vivo* secretion of human albumin (ALB) proteins in mouse blood were measured after the cell transplantation. As a result, human ALB amount in the blood of mouse transplanted with the hepatocyte-like cells could be detected after 1 week from transplantation (approximately 140 ng/ml). Moreover, the concentration of human ALB was increased to approximately 6.7 μg/ml after 4 weeks from transplantation. By contrast, human ALB amount in the blood of mouse transplanted with the DE cells and the hepatoblasts were hardly detected after 4 weeks from transplantation (below 103 ng/ml). The liver of the transplanted mouse was also analyzed by immunostaining and quantitative RT-PCR. Engraftment of the hepatocyte-like cells into the liver of uPA/SCID mice was evidenced by positive staining of human ALB and human CK8/18. In addition, the expression of human genes, such as *ALB*, *α1 anti-trypsin*, *HNF4α*, and *CYP7A1*, was observed only in the liver received the hepatocyte-like cells but not in those received the DE cells and hepatoblasts. In conclusion, the hepatocyte-like cells were much desirable for generation of human liver chimeric mice than the DE cells and hapatoblasts.

Intestinal/Gut Cells

W-1101

ADULT STEM CELLS IN THE SMALL INTESTINE ARE INTRINSICALLY PROGRAMMED WITH THEIR LOCATION-SPECIFIC DIFFERENTIATION FATE

Sabine Middendorp, Kerstin Schneeberger Schneeberger, Caroline L. Wiegerinck, Michal Mokry, Edward ES Nieuwenhuis
University Medical Center Utrecht, Utrecht, Netherlands

In mammals, the small intestinal epithelium is highly specialized along the cephalocaudal axis with different absorptive and digestive functions in duodenum, jejunum and ileum. Several transcription factors, like GATA4 and CDX2, have been described to regulate location-specific gene expression in the mouse small intestine. However, it is not known whether the intestinal environment, such as the mesenchyme, luminal content or the microbiota is necessary to maintain the location-specific functional properties of epithelial cells.
By using the organoid culturing technique, we cultured pure epithelial cells derived from location-specific crypts of mice and human to exclude the effect of extrinsic factors. We determined expression of location-specific genes, such as Gata4, disaccharidases and bile acid transporters. We show that the *ex vivo* expression signatures remained stable in location-specific mouse organoids that were expanded for up to 12 weeks. Furthermore, human duodenal and ileal organoid cultures that were expanded for 7 weeks and were then induced to differentiate, also maintained their functional identity corresponding with their original location.
These data show that within the small intestine, location-specificity is intrinsically programmed in the adult stem cells and is independent of extracellular signals from either mesenchyme or luminal content.

W-1102

TRANSCRIPTIONAL AND FUNCTIONAL DECLINE OF THE INTESTINAL STEM CELL NICHE DURING THE AGEING PROCESS

Christian M. Nefzger, Katja Horvay, Thierry Jarde, Jaber Firas, Helen E. Abud, Jose M. Polo
Anatomy and Developmental Biology, Monash University, Melbourne, Australia

Organ homeostasis and regeneration are facilitated by multipotent tissue stem cells that give rise to all the mature cell types of a specific organ. During an organism's life, adult stem cell pools are maintained by cell division requiring both the genome and the epigenome to be faithfully copied. However, according to the relatively few studies conducted to date, changes in the epigenome and in consequence gene expression can be detected in a large number of aged organs/tissues. The intestinal epithelium is an ideal model to study aging as it constitutes a high-turnover tissue that is renewed every 4-5 days. Epigenetic changes are likely to accumulate in the intestinal stem cell pool due to its high rate of cell division, and indeed age-related changes in DNA methylation status were shown for whole organ preparations. Coincidentally certain types of cancer (e.g. colorectal cancer) also increase with age, moreover alterations of the epigenome have been suggested to be the underlying causes. Age related changes also impact on functional properties of the intestinal epithelium as evidenced by a reduced capacity to take up essential nutrients like calcium and phosphorus. Combining fluorescent reporter genes and surface marker we have been able to extract intestinal stem cells (ISCs) in large numbers (up to 3*105 cells per animal). When embedded in matrigel intestinal crypts or single ISCs can be cultured in vitro and give rise to so called organoids, intestinal structures with crypt and villus-like epithelial domains that contain all differentiated types of cells. In order to study the effect of aging on the intestinal stem cell niche in detail we have performed immunohistochemical characterization of tissue sections, functional in vitro assays and expression profiling of the stem cell compartment in mice of different age groups (1.5 month, 10 month, 24 month). Our results showed clear changes in intestinal morphology, expression profile and regeneration potential. To further investigate the underlying causes for these functional and transcriptional changes, we are currently studying how different epigenetic modifications in the ISCs are altered during aging.

W-1103

FUNCTIONAL ANALYSIS OF SLC52A3, A TARGET GENE OF BROWN-VIALETTO-VAN LAERE SYNDROME

Atsushi Intoh, Kathryn Koszka, Naoki Suzuki, Nicholas Atwater, Kevin Eggan
Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States

Brown-Vialetto-Van Laere (BVVL) syndrome is a neural disease characterized by progressive ponto-bulbar palsy and bilateral sensorineural deafness. BVVL patients also have symptoms of lower motor neuron degeneration, muscle weakness, and amyotrophy. Diaphragmatic weakness is the most distressing feature in BVVL syndrome, leading eventually to respiratory failure. The prognosis for BVVL patients is poor, and most patients die within 10 years of onset. The cause of this disease had long remained unknown. However, mutations in the C20orf54 locus were recently identified in BVVL patients. C20orf54 encodes one member of riboflavin transporter family, and has a mouse homolog; Slc52a3. Riboflavin is a water soluble vitamin, widely known as vitamin B_2. Riboflavin is a precursor of FAD and FMD, which function as cofactors for a number of redox enzymes and play essential roles in the transfer of electrons in biological oxidation-reduction cycles. As humans and the other mammals cannot synthesize riboflavin *in vivo*, they must uptake this vitamin from their diet. However the association with neural differentiation or neurological system is still unknown.
Our question is that how the lack of this gene affects nerve systems in patients. To test for a function link between C20orf54/Slc52a3 and BVVL, we examined Slc52a3-KO mutant mice, embryos, and stem cell lines. As our results, Slc52a3 expresses in intestinal crypt and testis, but not in brain and spinal cord in adult mouse. The main function of Slc52a3 in adult mouse is expected as riboflavin absorption from the diet via intestinal epithelium. During mouse embryo development, Slc52a3 expresses in inner cell mass of blastocysts. In vitro experiments also showed that mouse and human embryonic stem cells have Slc52a3 expression. Moreover, Slc52a3 knock-out mice are em-

bryo lethality. These results suggest that failure of riboflavin uptake in adult intestine and during early development would affect BVVL syndrome.

W-1104

GENERATION OF STOMACH TISSUE FROM MOUSE EMBRYONIC STEM CELLS.

Takaaki K. Noguchi[1], Naoto Ninomiya[2], Makoto Asashima[3], Akira Kurisaki[1]
[1]Graduate School of Life and Environmental Sciences, The University of Tsukuba, Japan, Tsukuba city, Japan, [2]Department of Life Sciences (Biology), Graduate School of Arts and Sciences, The University of Tokyo, Japan, Meguro-ku, Japan, [3]Research Center for Stem Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST), Japan, Tsukuba city, Japan

Studies in embryonic development have led to successful efforts in differentiation research of mouse and human embryonic and induced pluripotent stem cells into specific organ cell types in vitro. For example, mouse embryonic stem cells have been differentiated into liver hepatocytes and pancreatic endocrine cells that have therapeutic efficacy in animal models of liver disease and diabetes, respectively. Compare to these organs, however, directed differentiation of stomach lineage from embryonic stem cells has not been reported and remains major challenge for translational studies in stomach disease model. Here, we established efficient differentiation method of mouse embryonic stem cells into stomach tissue. This process involves embryoid-body based intestinal gut-like structure differentiation method induced by sonic hedgehog (Shh) and Dickkopf-related protein 1 (Dkk1) that mimics embryonic intestine-stomach specification. These induced-stomach primordiums had Barx1-expressing stomach specific mesoderm and Sox2-expressing endoderm in three-dimensional structure imitates embryonic stomach tissue in vivo. Furthermore, induced-stomach primordiums could differentiate into three morphological stomach parts, forestomach, corpus, and antrum in three-dimensional pro-stomach culture system. The corpus region contained lots of H+/K+ ATPase-expressing parietal cells, and the antrum region contained lots of Pepsinogen-expressing chief cells in each stomach tissue part. Using this culture method as a model to study mouse stomach development and disease model, we identified that Shh induction and Wnt inhibiting are require for stomach specification and three-dimensional culture promotes stomach primordiums into mature stomach tissue in vitro. Lastly, we demonstarated that over expression of transforming growth factor-α (TGFα) increased the number of mucocus cells in stomach tissue using Rosa26 Tet-knock in system resembles " Menetrier diease" morphology in vitro. In conclusion, embryonic stem cells derived stomach tissue should allow for unprecedented studies of stomach development and human stomach disease model in the future.

W-1105

AN EPIGENETIC BASIS FOR LATERAL INHIBITION IN INTESTINAL STEM CELL DIFFERENTIATION

Tae-Hee Kim, Fugen Li, Isabel Ferreiro-Neira, Li-Lun Ho, Annouck Luyten, Kodanda Nalapareddy, Michael Verzi, Ramesh Shivdasani
Dana-Farber Cancer Institute, Boston, MA, United States

It is unclear, at the level of chromatin, if the potential to express diverse transcriptional programs is inherent in adult tissue stem cells or develops as they differentiate along distinctive lineages. In the process known as lateral inhibition, neighboring progenitor cells adopt two mutually exclusive fates: one cell, specified stochastically, transmits a signal that forces its neighbors to adopt a different identity. The two cell fates, however, remain interchangeable for long periods, implying chromatin plasticity at hundreds of genes. Intestinal stem cell (ISC) differentiation into enterocytes or secretory (goblet, endocrine, and Paneth) cells is attributed to Notch-mediated lateral inhibition among their immediate bipotential progeny. Expression of the transcription factor ATOH1 and Delta-like ligands in a few cells leads them to adopt the secretory fate and signal to Notch receptors in neighboring cells. This signaling activates HES1, a transcriptional repressor that prevents *Atoh1* expression and secretory differentiation in recipient cells, forcing the enterocyte fate. To investigate the epigenetic and transcriptional basis of this process, we purified Lgr5+ ISCs and absorptive and secretory progenitors from mouse intestinal crypts. mRNA expression profiles

of the isolated populations verified their respective potentials and identified lineage-specific genes. Genome-wide analysis of ATOH1 binding in secretory progenitors and *Atoh1*-dependent transcripts implicated this transcription factor in direct control of both lateral inhibition and secretory differentiation, through its binding to *cis*-elements of Delta-ligand genes and key lineage determinants. Despite showing large differences in expressed genes, absorptive and secretory progenitors carried almost identical marks of active chromatin - H3K4me2, H3K27ac, and DNaseI hypersensitivity, signifying nucleosome displacement -at most genes. Thus, despite cells' overt commitment to one branch of differentiation, their chromatin manifested the potential to activate the transcriptional program of either branch. As evidence for functionality of marked enhancers, depletion of ATOH1 in committed secretory cells *in vivo* was sufficient to convert them into enterocytes. Importantly, enhancers for genes restricted to each mature lineage were well-delineated and showed early signs of activation in Lgr5+ ISCs, generating a broadly permissive chromatin state. Thus, lateral inhibition in the intestine is driven by direct activity of a lineage-specific transcription factor operating in a permissive chromatin state that is established in multipotent stem cells. These findings explain a fundamental differentiation mechanism with respect to chromatin states and elucidate the basis for lineage plasticity in ISCs and their immediate progeny.

W-1106

PRETREATMENT WITH TLR LIGANDS ENHANCES THE IMMUNOSUPPRESSIVE CAPACITY OF MESENCHYMAL STEM CELLS IN ANIMAL MODEL OF PROCTITIS

Christine Linard, Jean Victor Lacave-Lapalun, Marc Benderitter
IRSN, Fontenay aux roses, France

Augmenting tissue repair by the use of mesenchymal stem cells (MSCs) may be an important advance in treating radiation-induced proctitis. The immunosuppressive capacity of MSCs was become a key factor for their therapeutic use and potency. Recently reported that TLR ligands can improve the MSCs capacity to suppress immune responses and repair. Using a colorectal model of 27Gy irradiation in rats, we investigated the effects of *i*) systemic MSCs (5.10^6) administration (rat MSCs) *ii*) TLR5 ligand flagellin administered (3 days post-irradiation) prior the systemic MSCs administration (rat flagellin pre-treatment) or *iii*) MSCs preconditioned 24h in culture with flagellin (rat Flag-MSC). MSCs or Flag-MSCs were administered 7 days post-irradiation. At 28 days post-irradiation, histopathological lesions are similar to those seen clinically (severe acute mucosal ulceration, large immune cell infiltration. Flow cytometric analysis of isolated colonic immune cells population showed that MSCs, Flag-MSC treatment and MSCs injected after flagellin pre-treatment reduced significantly the innate immunity response (neutrophil and macrophage infiltration) as compared to irradiated rats. Although the among of natural killer cells was not decrease by MSCs, Flag-MSC and flagellin pre-treatment, RT-PCR assays showed that Flag-MSC and flagellin pre-treatment induced INOS overexpression, crucial to achieve immunosuppression. Analysis of the $CD4^+$ and $CD8^+$ T cells frequency revealed that MSCs, Flag-MSCs and flagellin pre-treatment did not modified the enhanced $CD4^+$ frequency in the irradiated colonic mucosa; on the other side the CD4/CD8 ratio decreased with MSCs, increase with flagellin pre-treatment and, in opposite was normalized with Flag-MSCs compared to irradiated rats. Surprisingly, in the irradiated colonic mucosa no modification of the suppressive T-cell population Treg ($CD4^+CD25^+$) was induced by MSCs alone as compared to irradiated rats, but Flag-MSC and flagellin pre-treatment overexpressed FOXP3 and IL-2Rα which characterized an enhanced functional Treg phenotype. Specifically, IL-10 was overexpressed with Flag-MSCs. In addition, MSCs, Flag-MSCs and flagellin pretreatment, in the same manner, restored the epithelial cell proliferation (characterized by Ki67 overexpression) and the antimicrobial Reg3γ protein implicated in host defense. In conclusion, on a proctitis model, MSCs exposed to activation through TLR5 ligand (pre-activation of the host or pretreated-MSCs) have an enhanced immunosuppressive capacity.

W-1107

A HUMAN IPSC MODEL OF FAMILIAL ADENOMATOUS POLYPOSIS

Cesar A. Sommer, Andreia Gianotti Sommer, Gustavo Mostoslavsky

Center for Regenerative Medicine (CReM), Boston University School of Medicine, Boston, MA, United States

Mutation of the Adenomatous Polyposis Coli (APC) tumor suppressor gene underlies Familial Adenomatous Polyposis (FAP) and is an early event in most colorectal adenomas and carcinomas. APC is a multidomain protein controlling fundamental cellular processes that include intestinal cell migration and proliferation, spindle formation, and chromosome segregation. We developed an in vitro model of FAP by generating induced pluripotent stem cells (iPSCs) from fibroblasts isolated from individuals carrying germline mutations in APC. The FAP-iPSC lines were induced to form intestinal tissue by directed differentiation, resulting in 'intestinal organoids' resembling normal intestinal epithelium with expression of typical gut markers. Cell proliferation, Wnt target gene expression and cell polarity assays were performed in iPSCs and their differentiated progeny. To investigate the functional effects of biallelic APC inactivation in FAP-IPSCs, we designed and assembled Transcription Activator-Like Effector Nucleases (TALENs) targeting codon 1309 in the mutation cluster region of APC. Our results provide a platform for elucidating disease mechanisms underlying FAP with possible future applications in high-throughput drug screening.

Lung Cells

W-1111

BONE MARROW CELLS EXPRESSING CLARA CELL SECRETARY PROTEIN ARE CAPABLE OF FORMING FUNCTIONAL CONNEXIN CHANNELS WITH MATURE EPITHELIAL CELLS.

Paula Marcus, Pascal Duchesneau, Rickvinder Besla, Thomas K. Waddell
Latner Thoracic Surgery, UHN, Toronto, ON, Canada

Background: Bone marrow (BM) may contain populations of stem and progenitor cells required for the repair of many tissues. Previous data has indicated the presence of a population of bone marrow cells that express the airway marker Clara cell secretary protein (CCSP) and that these cells are capable of re-populating the airway after injury. However, the exact role these cells in repair is unclear. The aim of this work is to determine if the CCSP+ BM cells are capable of communicating with mature epithelial cells via the formation of connexin (Cx) channels.
Materials and methods: BM cells expressing CCSP were isolated from C57Bl/6 mice by flow cytometry after 7 days in culture. The expression of airway related Cx molecules (Cx 43, 32 and 26) was determined through quantitative PCR (qPCR). The presence of Cx channels *in vivo* was examined by immunofluorescence microscopy following transtracheal delivery of CCSP+ BM cells in a mouse model of airway injury naphthalene. Finally, the presence of functional communication was evaluated by labelling CCSP+ cells with the membrane dye PKH26
(Sigma) and calcein-AM (Invitrogen), a dye that can be transferred through Cx channels. Labelled cells were either mixed with the bronchial epithelial cell line Beas-2b, or delivered intratracheally to 8 week old mice. To ensure specificity of dye transfer, controls where Cx channels were blocked with carbenoxolone (Sigma) were included.
Results: CCSP+ BM cells expressed epithelial specific Cx molecules,
and stained positively for Cx43. The CCSP+ BM cells were retained in the airway after delivery into a naphthalene injured mouse. These cells appeared capable of forming connexin channels with airway cells, as indicated by Cx43 labelling at the periphery of the delivered cells. However, to determine if the CCSP+ BM cells could communicate we looked at dye transfer between epithelial cells and the BM cells. Dye transfer occurred *in vitro* between the two populations, and this transfer could be reduced with the addition of a gap junction block. Preliminary data also suggests that these CCSP+ cells are capable of transferring dye *in vivo* to airway cells as early as 4 hours after delivery.
Conclusions: CCSP+ BM cells are capable of expressing the required elements for gap junction communication, both *in vitro* and *in vivo*. Moreover, the cells can also form functional Cx channels with mature epithelial cells, as indicated by the ability to transfer dye. This suggests that the CCSP+ BM cells are able to interact with epithelial cells within the airway after injury, and it is possible that the formation of these channels is important in the repair response.

W-1112

GENERATION OF AIRWAY AND LUNG EPITHELIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Sarah Xuelian Huang, Ya-wen Chen, Melanie Mumau, Hans-Willem Sneock
Medicine/CCTI, Columbia University, New York, NY, United States

Generation of airway and lung epithelial cells from human pluripotent stem cells

Sarah X.L. Huang[1], Ya-Wen Chen[1], Melanie Mumau[1], Hans-Willem Snoeck[1]
[1]Columbia Center for Translational Immunology, Columbia University Medical Center, New York, NY, USA

Rationale: Directed differentiation of human pluripotent stem cells (hPSCs) *in vitro* towards lung progenitors, using a defined combination of morphogens, following developmental paradigms, should be able to recapitulate lung development *in vivo*, allow generation of human lung and airway epithelial cells with high efficiency.

Significance: Efficient directed differentiation of hPSCs into most types of lung and airway epithelial cells has not been achieved thus far[1-3]. Yet, the ability to generate such cells from hPSCs has applications in regenerative medicine for lung diseases, drug screening and disease modeling, and provides a model to study human lung development[4].

Aims:

1. To generate early human lung progenitors from hPSCs with high purity.
2. To further mature the early lung progenitors into a variety of airway and lung epithelial cells that express lineage-specific markers.

Methodology: The aims were achieved through a strategy involving hierarchal, sequential, and stepwise induction of definitive endoderm[5], followed by generation of anterior foregut endoderm[4] that is specified with high efficiency towards $NKX2.1^{+}FOXA2^{+}$ developmental lung field progenitors, and subsequent long-term differentiation into cultures where most markers of lung and airway epithelial cells are expressed in varying ratios. The expression of lineage specific phenotypic markers at each differentiation stage was examined at both mRNA and protein levels.

Results: We developed, based on developmental paradigms, a highly efficient method for directed differentiation of hPSCs into early lung progenitors where ~95% of the cells were $FOXA2^{+}$, and where 80-90% of $FOXA2^{+}$ cells expressed NKX.2.1 (Fig. 1a).

Prolonged culture of the early lung progenitors until d48 induced most markers of airway and respiratory epithelial cells. These markers included mucins (MUC1, MUC5AC, MUC2, MUC5B) (Goblet cells), ample expression of CC10 (Clara cells), a secreted protein that was also detected outside of cell boundaries, FOXJ1 (ciliated cells), proSP-C and SP-B (ATII cells) as well as AQ5 and PDN (putative markers of ATI cells) (Fig. 1b).

Conclusions: This report is the first to show full differentiation of hESCs into a wide array of lung and airway cells, such that most cells in the culture could be assigned to a type of respiratory epithelial cell. Currently, we are trying to develop assays/models to test the function of the lung and airway epithelial cells.

W-1113

MESENCHYMAL STEM CELLS STIMULATE BRONCHIOALVEOLAR STEM CELL DIFFERENTIATION

Kristen T. Leeman[1], Carla Kim[2]
[A]Department of Newborn Medicine, [B]Department of Hematology Oncology, [1]Boston Children's Hospital, Boston, MA, United States

Background: Bronchopulmonary dysplasia (BPD) remains a major complication of prematurity resulting in significant mortality. BPD includes alveolar epithelial simplification and distal lung injury. Treatment with bone marrow derived mesenchymal stem cells (MSC) and MSC-conditioned media ameliorated lung parenchymal and vascular injury in the hyperoxia murine model of BPD. It is possible that the beneficial response from the MSCs is partly due to activation of endogenous lung epithelial stem cells. Bronchioalveolar stem cells (BASCs) are an adult lung stem cell population capable of self-renewal and differentiation in culture, and BASCs proliferate in response to bronchiolar and alveolar lung injury in vivo. Systemic treatment of neonatal hyperoxia-exposed mice with MSCs or MSC-CM significantly increased BASCs compared to untreated controls. Treatment of BASCs with MSC-CM in 2D culture showed an increase in growth efficiency, indicating a direct effect of MSCs on BASCs.

Hypothesis: We hypothesize that distal lung stem cells contribute to repair and regeneration in neonatal lung injury.
Design/Methods: We established a new 3D matrigel assay to assess BASC differentiation potential after exposure to MSCs. FACS isolated BASCs (CD31-CD45-EpCAM+Sca1+ cells) from β-actin GFP mice were mixed with growth factor reduced matrigel and MSCs or control stromal cells, lung primary mouse endothelial cells (MECs). Cell mixtures were plated on transwell inserts and standard media was added to the bottom of the plate. 14 days after plating, cultures were examined for heterotopic
colony formation including bronchiolar colonies, alveolar colonies, and colonies with mixed phenotype, the later of which retain progenitor activity. Hematoxylin and eosin (H&E) staining and immunofluorescence for CCSP and SPC confirmed colony differentiation type.
Results: MSCs significantly increased BASC colony numbers 2.8-fold compared to co-culture with MECs (p=0.04). Colonies arising in MSC co-culture were significantly larger compared to controls; 41% of colonies with MSCs were large or medium vs 22% in controls. These data support our hypothesis that MSC-secreted factors enhance BASC function. BASC/MSC co-cultures trended towards more bronchiolar and alveolar colonies compared to BASC/MEC cultures with more prevalent mixed phenotype colonies. This may indicate MSC factors stimulate BASC proliferation and differentiation, whereas MEC factors foster maintenance of progenitor cells.
Conclusions: BASCs may play a role in the repair of alveolar lung injury in BPD and in the restoration of distal lung cell epithelia. MSCs and MSC-derived factors may stimulate BASC differentiation and enhance repair of neonatal lung injury.

W-1114

EFFECTS OF FIBROBLAST GROWTH FACTOR 9 ON STEM CELLS OF UPPER AIRWAYS

Ahmed E.M. Hegab[1], Daisuke Arai[1], Kenzo Soejima[1], Steven L. Brody[2], David M. Ornitz[2], Tomoko Betsuyaku[1]
[1]Keio University School of Medicine, Tokyo, Japan, [2]Washington University School of Medicine, St. Louis, MO, United States

RATIONALE:
Many of the fibroblast growth factors (FGFs) are reported to promote self-renewal, proliferation and inhibition of cellular senescence mainly through effect on tissue-associated stem cells. FGF-9 is a secreted signaling molecule that is required for lung development and is highly expressed in a subset of human lung adenocarcinomas. However, specific function of FGF-9 and its effect on adult tracheal basal stem cells are unknown.
METHODS:
We used both two- and three-dimensional primary culture systems, the air-liquid interface (ALI) and stem cell sphere-formation system, respectively, to examine the effect of FGF9 treatment on the behavior of mouse tracheal epithelial cells (mTEC). In both assays, cells were treated with FGF9 10-300 ng/ml for 1-3 weeks. ALI preparations were assayed for cell proliferation and differentiation by immunostaining. Stem cell spheres were assayed for sphere number, size, differentiation and self-renewal.
RESULTS:
Immunostaing of ALI preparations for tracheal epithelial cell and proliferation markers (cytokeratin 5 and TRP63 for basal cells, CCSP for Clara cells, acetylated β-tubulin for ciliated cells and PCNA to detect proliferating cells) revealed that FGF9 induced the rate of basal cell proliferation in a dose-dependent fashion. Low and medium doses of FGF9 treatment did not affect differentiation of basal cells into Clara and ciliated cells while high dose FGF9 resulted in an increase in basal and a decrease in Clara cells; i.e., basal cell hyperplasia. FGF9 treatment significantly increased average sphere diameter and the number of spheres per well, compared to control treatment, indicating potentiation of stem cell self-renewal and increase activation or decrease senescence of stem cells.
CONCLUSION:
FGF9 accelerates the self-renewal of basal stem cells and in higher doses suppress their differentiation into the secretory lineage. This finding suggests a potential role of FGF9 in repair or tumorigenesis in adult lungs.
Funding source: Scientific Research Grants from the Ministry of Education, Science, Culture and Sports of Japan, 23390218

W-1115

ABROGATION OF SIX1/EYA1 DISRUPTS THE SACCULAR PHASE OF LUNG MORPHOGENESIS AND CAUSES REMODELING

Karol Lu[1], Raghava Reddy[1], Barbara Driscoll[2], David Warburton[2], Ahmed El-Hashash[2]
[1]Children's Hospital Los Angeles (CHLA), Los Angeles, CA, United States, [2] Children's Hospital Los Angeles (CHLA) and University of Southern California, Los Angeles, CA, United States

The Eya1 gene encodes a transcriptional co-activator that acts with Six1 gene to control the development of different organs. However, Six1-Eya1 genetic interactions and functional roles in lung remodeling in premature infants with bronchopulmonary dysplasia (BPD) are still unknown.
Objective: Determine the functional role of Six1-Eya1 genetic interactions in lung remodeling in premature infants with bronchopulmonary dysplasia (BPD).
Hypotheses: Based on the premise that Eya1 and Six1 genes regulate development of different organs and differentiation of various progenitor cells, we assume that Six1-Eya1 genetically interacts to synergistically regulate lung morphogenesis, and their loss results in a pre-BPD-like saccular phenotype. We further postulate that TGF-β signaling pathway is involved in the development of pre-BPD-like saccular phenotype.
Materials and Methods: Lungs of compound homozygous (Eya1-/- or Six1-/-) and heterozygous mice and histological sections of E14.5 and E18.5 were used to examine phenotypic characteristics. The relationship between Eya1 and Six1 was determined by co-immunoprecipitation, Western blot assays and in vitro knockout of Six1 followed by overexpression of Eya1 in MLE15 cells.
Results: We found that pathological changes in Eya1-/- or Six1-/- mice bore similarities to premature infants born in the saccular phase who develop BPD, including mesenchymal thickening, remodeling of the distal lung septae and arteries as well as abnormal capillary development. Thus, mutant lung histology at the saccular phase shows abnormal lung morphogenesis, characterized by mesenchymal and saccular wall thickening, abnormal proliferation of α-smooth muscle actin-positive cells, increased mesenchymal/fibroblast cell differentiation and poor capillaries development, which become more severe when deleting both genes.
Conclusion: Our study indicates that SHH but not TGF-β signaling pathway is a central mediator for the histologic alterations described in association with pre-BPD-like saccular phenotype in Eya1-/- or Six1-/- lungs. Indeed, genetic reduction of SHH activity in vivo or inhibiting its activity in vitro substantially rescues the pre-BPD-like phenotype in mutant mice at the saccular phase, providing the first evidence for the potential involvement of SHH signaling in BPD. These findings uncover novel functions for Six1-Eya1-SHH pathway in the development of BPD-like saccular phenotype, providing a conceptual framework for future mechanistic and translational studies in this area.

Epidermal Cells

W-1121

INTRADERMAL ADIPOCYTES MEDIATE FIBROBLAST RECRUITMENT DURING SKIN WOUND HEALING

Barbara Schmidt, Valerie Horsley
MCDB, Yale University, New Haven, CT, United States

The communication of multiple cell types in the skin is essential for epidermal and dermal repair following acute wounding. Interaction between hematopoietic cells, keratinocytes, and fibroblasts has been studied extensively during skin wound healing, as these cell types must proliferate and migrate to restore a functional skin barrier. However, the possible roles for other cell types within the skin, such as the intradermal adipocytes, have not been investigated during skin regeneration. Here, we present data demonstrating that adipocyte lineage cells are activated and repopulate skin wounds after injury. We find that adipocyte repopulation occurs after the immune response and in parallel with fibroblast migration. Functional analysis of mice with defects in adipogenesis demonstrates that adipocytes are necessary for the recruitment of fibroblasts into the wound bed and subsequent reconstruction of the dermis. Interestingly, these data reveal that initial re-epithelialization can occur in the absence of

fibroblast recruitment but cannot be maintained, resulting in wound failure. These data implicate adipocytes as a key component of the intercellular communication that mediates fibroblast function during skin wound healing.

W-1122

INDISPENSABLE ROLES OF BNIP3, AN INDUCER OF AUTOPHAGY, IN BOTH DIFFERENTIATION AND MAINTENANCE OF EPIDERMAL KERATINOCYTES.

Mariko Moriyama, Junki Uda, Hiroyuki Moriyama
Kinki University, Higashi-Osaka, Japan

The skin epidermis is a stratified epithelium. Stratification is a key process of epidermal development. During epidermal development, the single layer of basal cells undergoes asymmetric cell division to stratify, and produce committed suprabasal cells on the basal layer. These suprabasal cells are still immature, and sustain several round of cell divisions to form fully stratified epithelia. Recent studies have clarified a numerous number of molecules involved in epidermal development, although it remains elusive how these molecules are coordinated to undergo proper stratification of the epidermis. Autophagy, a lysosomal degradation pathway, is involved in differentiation of erythrocytes, lymphocytes, and adipocytes. Keratinocyte differentiation is also going along with activation of lysosomal enzymes and organelle clearance, expecting the contribution of autophagy in this process.
Previously, by integrating both loss- and gain-of-function studies of Notch receptors and their downstream target Hes1, we show multiple roles of Notch signaling in the regulation of transit amplifying cells in suprabasal layers. Notch signaling induces differentiation of suprabasal cells via Hes1 independent manner, whereas Hes1 is required for maintenance of the immature status of suprabasal cells by preventing premature differentiation. In this study, we found that Hes1 directly suppressed the expression of Bnip3, whose expression is sufficient to induce terminal differentiation of keratinocytes by induction of autophagy.
Chromatin immunoprecipitation assay revealed that HES1 could directly bind to *BNIP3* promoter to suppress the expression. We also found that BNIP3 was expressed in the granular layers, just above the layers where Hes1 expression was observed. Consistent with the BNIP3 expression, autophagosome formation was observed in the granular layer of human epidermal equivalent reconstituted from GFP-LC3 expressing keratinocytes. Forced expression of BNIP3 in human primary epidermal keratinocytes (HPEK) resulted in keratinocyte differentiation, whereas knockdown of BNIP3 had an opposite effect. Intriguingly, addition of an inhibitor of autophagy, 3-methyladenine, significantly suppressed the BNIP3-stimulated differentiation of keratinocytes, suggesting that autophagy is involved in this process. Moreover, we also found that overexpression of BNIP3 induced autophagy in HPEK. These data clearly suggest that BNIP3 plays a crucial role in keratinocytes differentiation by inducing autophagy. Furthermore, dead cells were increased in human epidermal equivalent from BNIP3 knockdown keratinocytes, which gave us the idea that BNIP3 is also indispensable for maintenance of skin epidermis. To test the hypothesis, HPEK were irradiated with UVB. UVB irradiation stimulated BNIP3 expression and cleavage of caspase3. Surprisingly, suppression of BNIP3 expression induced by UVB irradiation caused a further increase of the cleaved caspase3 protein level, suggesting that BNIP3 has a protective effect against UVB-induced apoptosis in keratinocytes. Overall, our data shed light on functions of BNIP3, an inducer of autophagy, in both differentiation and maintenance of epidermal keratinocytes.

W-1123

TOWARDS UNDERSTANDING THE ROLE OF HOXB7 IN THE SKIN

Fadi P. Jacob, Aiko Sada Nishimura, Tudorita Tumbar
Molecular Biology and Genetics, Cornell University, Ithaca, NY, United States

Epidermal stem cells are known to exist in a specialized niche of the hair follicle known as the bulge, but whether or not all cells of the bulge are of equal potential still remains an important question.
Microarray data displayed clear differences in gene expression between bulge and non-bulge cells, and Hoxb7 was among the genes up-regulated in the bulge. Hoxb7 is a sequence-specific transcription factor of the homeobox fam-

ily that is involved in cell proliferation and differentiation, and has known functions in the developmental regulatory system that provides cells with specific positional identities on the anterior-posterior axis. To investigate the potential role of Hoxb7 in epidermal tissue, we analyzed Hoxb7 expression and lineage activity using two transgenic mice: a Hoxb7-IRES-GFP reporter strain and a Hoxb7-cre/Rosa26-LacZ strain where cre activity in Hoxb7-expressing cells enabled lacZ reporter expression not only in cells that express Hoxb7, but also in their progeny. Surprisingly, we were unable to detect GFP expression in the skin epidermis as we expected from our microarray results and QRT-PCR data for Hoxb7 mRNA levels. Instead, the Hoxb7-IRES-GFP mice showed bright GFP expression in the dermal papilla of the hair follicles and dim GFP in dermal cells. The dermal cells surrounding the hair follicle, especially around the bulge were brighter than the dermal cells located elsewhere. This suggested that the Hoxb7 promoter region utilized for this transgenic might be insufficient to fatefully recapitulate Hoxb7 endogenous expression in the skin, and was potentially influenced by chromosome insertion position effects. The Hoxb7-cre/Rosa26-LacZ mice were analyzed at various stages in embryonic development, as well as in newborn and adult mice during the telogen, anagen, and catagen phases of the hair cycle. An interesting striped pattern of lacZ expression emerging at E14.5 on the back skin was observed in whole-mount tissue. In tissue sections, lacZ expression was detected in several patches of the skin and in various areas of the hair follicle depending on the cycle. Expression in the bulge, as well as other areas of the hair follicle at telogen, followed by expression in patches of the anagen follicle suggested that stem cells in the bulge of some follicles were labeled by the reporter system at some point in development. We hypothesize based on this data that Hoxb7 may play a role in skin patterning and proliferation.

W-1124

THE INTERFOLLICULAR EPIDERMIS HOMEOSTASIS IS MAINTAINED BY A HAIR FOLLICLE DERIVED STEM CELL IN A HIERARCHICAL MANNER

Edwige Roy[1], Zoltan Neufeld[2], Luca Cerone[2], Angeline Wong[1], Samantha Hodgson[1], Inna Tabansky[3], Kevin Eggan[3], Jean Livet[4], **Kiarash Khosrotehrani**[1]

[1]Experimental Dermatology Group, University of Queensland Centre for Clinical Research, Brisbane, Australia, [2]Department of mathematics and physics, University of Queensland, Brisbane, Australia, [3]Dept of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States, [4]Institut de la Vision, Paris, France

Despite the success of epidermal stem cell therapy, many questions remain on their true potency in steady state conditions. Indeed, the maintenance of the interfollicular epidermis (IFE) remains a controversial subject. Conflicting studies support the existence a stem cell hierarchy in the IFE or a committed progenitor activity without a specific hierarchy. However, recent results revealed a second population of slow-cycling stem cells besides CPs. We hypothesized that those conflicting studies could be resolved using the rainbow technology to label keratin 14-expressing keratinocytes. We generated transgenic mice containing a Brainbow-1.0 cassette under the control of a ubiquitous promoter allowing the random expression of dTomato (red), Cerulean (blue) or eYFP (yellow) upon cre recombination. When crossed with K14-Cre/Esr transgenic mice, this allowed us to induce recombination in all basal keratinocytes regardless of their anatomical location and to follow their fate over time.
To address the role of stem cells, we conducted short and long term experiments at 3, 5, 12 and 24 weeks. Clones within the IFE of dorsal skin grew in size from 3 weeks to 6 months ($p<0.001$) in accordance with the committed progenitor model. To understand the lineage relationship between the CP and these potential stem cells, we analysed the size of clones relative to their positioning and we observed those that were attached or contained a hair follicle were significantly larger ($p<0.05$) and grew more steadily overtime ($p<0.0001$).
Further investigations showed that clones in areas of active hair cycling were larger compared to clones not attached to HF ($p<0.05$) and that proliferation in the HF infundibulum was hair cycle dependent and occurred mainly in anagen ($p<0.0001$) suggesting that this hair cycle phase promoted the growth of IFE clones via a HF contribution. We proposed a model where stem cell populations in HF generated CPs around them which behaved with random fate decision. Using this model, computer simulations integrating 6 colour staining and animal growth predicted clone sizes that closely matched our observations.

In conclusions, Rainbow technology allows the long term tracking of clones derived from individual stem cells. Our results support a hierarchical contribution of hair follicle stem cells to dorsal IFE by differentiating into committed progenitors. Our findings and our proposed model reconcile recent and previous studies showing the importance of the upper portion of the HFs for IFE homeostasis in murine dorsal skin.

W-1125

BAF53A ENFORCES THE EPIDERMAL PROGENITOR STATE BY SUPPRESSING SWI/SNF-DEPENDENT INDUCTION OF KLF4

Xiaomin Bao[1], Jiong Tang[2], Vanessa Lopez-Pajares[1], Shiying Tao[1], Kun Qu[1], Gerald Crabtree[2], Paul Khavari[1]
[A]Dermatology, [B]HHMI, [1]Stanford University, Stanford, CA, United States

Somatic progenitors suppress differentiation to maintain tissue self-renewal. While epigenetic regulators of DNA and histone modifications can support such repression, a role for nuclear actin-like proteins, such as ACTL6a/BAF53A, is unclear. Here we show that ACTL6a associates with the SWI/SNF chromatin-remodeling complex to suppress differentiation in epidermis. ACTL6a was found enriched in progenitors and down-regulated during differentiation. ACTL6a loss in murine and human tissue abolished epidermal self-renewal and induced terminal differentiation, whereas ectopically expressed ACTL6a suppressed differentiation. Among known activators of epidermal differentiation, KLF4 controls 48.6% of ACTL6a regulated genes. ACTL6a loss upregulated KLF4 and dysregulated KLF4's target genes, effects that were blocked by KLF4 depletion. Furthermore, we show that the SWI/SNF complex regulates KLF4 activation and differentiation, and ACTL6a loss led to enhanced SWI/SNF binding to the promoters of KLF4 and other differentiation genes. ACTL6a thus maintains the undifferentiated progenitor state, partly by suppressing SWI/SNF complex-enabled induction of KLF4.

W-1126

SYNCHRONIZING EFFECT OF CELL-AUTONOMOUS CIRCADIAN CLOCK ON CELL CYCLE IN TRANSIENT AMPLIFYING EPITHELIAL PROGENITORS OF HAIR FOLLICLE

Maksim Plikus[1], Christopher Vollmers[3], Amandine Chaix[3], Damon de la Cruz[4], Raul Ramos[2], Satchidananda Panda[3], Cheng-Ming Chuong[4]
[A]Developmental and Cell Biology, [B]Department of Developmental and Cell Biology, Sue and Bill Gross Stem Cell Reseach Center, [1]University of California, Irvine, Irvine, CA, United States, [2]Salk Institute for Biological Studies, La Jolla, CA, United States, [3]Pathology, University of Southern California, Los Angeles, CA, United States

Circadian clock optimizes various physiological processes through their coordination with the rhythmic changes in the environment. Despite significant advances in the field of clock research, circadian regulation of tissue regeneration is not well understood. Hair follicle stands out as a leading example of an organ capable of continuous physiological regeneration. Each hair follicle cycles through the sequential phases of growth, involution and inactivity. Successful progression through these phases requires complex coordination of the epithelial lineage, from stem cells to terminally differentiated keratinocytes. Recently, circadian clock was implicated in regulation of hair follicle stem cells.
Here we explore the role of circadian clock in the highly proliferative transient amplifying hair follicle progenitors, aka matrix cells. We show that in hair matrix cell-autonomous clock generates daily mitotic rhythms, so that numbers of mitotic cells peak in the morning. This cell cycle regulation is accomplished via circadian synchronizing of Cdc2/Cyclin B-mediated G2/M checkpoint. As the result, mouse hairs grow faster in the morning. During morning hours they are also much more sensitive to genotoxic stress, such as gamma-irradiation. Same doses of gamma-irradiation result in significantly greater damage to transient amplifying matrix population and cause more dramatic hair loss in the morning than in the evening.

These results demonstrate how circadian mitotic gating becomes an essential protective mechanism for the highly proliferative subset of cells within regenerating hair follicles. Furthermore, our results can offer new strategies for minimizing or maximizing cytotoxicity of radiation and chemo cancer therapies.

W-1127

THE ANDROGEN RECEPTOR MODULATES MURINE EPIDERMAL STEM CELL PLASTICITY AND HAIR FOLLICLE LINEAGE FATE

Kai Kretzschmar[1], Denny L. Cottle[2], Pawel J. Schweiger[2], Fiona M. Watt[1]
[1]Centre for Stem Cells and Regenerative Medicine, King's College London, London, United Kingdom, [2]Cambridge Stem Cell Institute, University of Cambridge, Cambridge, United Kingdom

Murine epidermis is composed of three main compartments: interfollicular epidermis, sebaceous glands (SGs) and hair follicles (HFs). Previous work has established that distinct stem cell pools are responsible for maintaining homeostasis of these three compartments during adult life. However, during tissue regeneration or upon genetic manipulation these stem cell populations are able to replenish each other. To study the ability of epidermal stem cells to differentiate into alternative lineages, we have previously described a transgenic mouse model (deltaK5deltabeta-cateninER), in which ectopic hair follicles (EFs) are selectively induced from the SG upon experimental activation of beta-catenin. We now found that beta-catenin induced EF formation in the SG can be blocked through AR activation, which preserves sebocyte fate. Conversely, AR inhibition enhances conversion to the HF lineage. Interestingly, in pre-existing HF simultaneous beta-catenin activation and AR inhibition caused the development of HF cysts resembling HF tumours. Taken together our results indicate that the AR modulates epidermal stem cell plasticity and hair follicle lineage selection.

W-1128

UNRAVELING THE IMPACT OF IL-1 SIGNALING ON STEM CELL ACTIVATION IN A WOUND HEALING MODEL

Colin Jamora[1], Pedro Lee[2], Deborah Witherden[3], Eve Kandyba[5], Amanda MacLeod[7], Krzysztof Kobielak[6], Wendy Havran[4]
[1]IFOM-inStem Joint Research Laboratory, Institute for Stem Cell Biology and Regenerative Medicine, Bangalore, India, [2]Biological Sciences, University of California, San Diego, La Jolla, CA, United States, [A]Immunology and Microbial Sciences, [B]Immunology and Microbial Science, [3]The Scripps Research Institute, La Jolla, CA, United States, [A]Pathology, [B]Department of Pathology, [4]University of Southern California School of Medicine, Los Angeles, CA, United States, [5]Immunology and Microbial Sciences, The Scripps Research Insitute, La Jolla, CA, United States

The wound healing response is the product of a complex network of intercellular communications that are orchestrated to repair the damaged tissue. A fundamental process mediated by these reciprocal interactions is the mobilization of local stem cells pools to promote tissue regeneration and repair. Using the epidermal caspase-8 conditional knockout as a genetic model of wound healing, we uncovered different modes by which interleukin-1α (IL-1α) rouses the proliferation of stem cells within distinct niches in the skin. Upon release from epidermal keratinocytes in response to a wound, IL-1α is sufficient to trigger dermal fibroblasts to preferentially activate epidermal stem/progenitor cells. In combination with IL-7, it can also stimulate resident dendritic epidermal T cells (γδT-cells), which favor hair follicle stem cell proliferation. Altogether these finding demonstrate the mechanism by which IL-1 signaling marshals multiple cell types to differentially stimulate distinct epithelial stem cell populations in the skin to restore tissue homeostasis.

Epithelial Cells (Not Skin)

W-1132

RARE SOMATIC CELLS FROM HUMAN BREAST TISSUE EXHIBIT EXTENSIVE LINEAGE PLASTICITY

Somdutta Roy, Phillipe Gascard, Thea Tlsty
Univ of California San Francisco, San Francisco, CA, United States

Extensive studies have demonstrated that repression of $p16^{INK4a}$ (CDKN2A) not only silences a powerful tumor suppressor activity, but also is associated with the acquisition of a plastic state, i.e. the ability of a cell to change phenotypes. In epithelial cells, repression of $p16^{INK4a}$ does not only inactivate cell cycle arrest in response to stress but also allows increased expression of chromatin remodeling proteins that are important for epigenetic plasticity underlying differentiation. The up-regulation of such chromatin remodeling proteins sets the expression pattern of pluripotent cells in Drosophila and inhibits differentiation and dictates the decision between progenitor and differentiated states in murine myoblasts. Furthermore, mice engineered for knock-out of BMI-1, a polycomb protein that inhibits $p16^{INK4a}$ transcription, fail to repress $p16^{INK4a}$ activity and fail to generate hematopoietic and neural stem cells. In light of these observations, we reasoned that repression of $p16^{INK4a}$ might also modulate expression of cell surface markers that could be used for the prospective isolation of stem or progenitor cells.

We identified cell surface markers that allowed direct isolation of rare cells from healthy human breast tissue that exhibit extensive lineage plasticity. This subpopulation has the potential to transcribe plasticity markers, Oct3/4, Sox2 and Nanog at levels similar to those measured in human embryonic stem cells. *In vitro*, *in vivo* and teratoma assays demonstrated that either a directly-sorted (uncultured) or a single cell (clonogenic) cell population from primary tissue can differentiate into functional derivatives of each germ layer, ectodermal, endodermal and mesodermal. In contrast to other cells that have the potential to express Oct3/4, Sox2 and Nanog, these human endogenous Plastic Somatic cells (ePS cells) are mortal, express low telomerase activity, expand for an extensive but finite number of population doublings, and maintain a diploid karyotype before arresting in G1.

Cells with extensive lineage plasticity exist in healthy tissues and evidence for their plasticity is sometimes observed by pathologists in non-diseased tissue. While the vast majority of stem cell/stromal interactions generate the expected tissues, in rare instances, the signals are misconstrued or malfunction. These rare events, described by pathologists as ectopic tissues, consist of normal tissues in abnormal locations, such as bone in the colon and eye, liver in gallbladder and pancreas in brain.

We anticipate that the novel somatic cell population with extensive lineage plasticity characterized here may be used in the future as a non-embryonic resource to study acquisition of pluripotency, self-repair, replacement and complications in regenerative medicine and cancer.

W-1133

A NOVEL AT-HOOK CONTAINING TRANSCRIPTION FACTOR PROMOTES DIFFERENTIATION OF NEURAL STEM CELLS.

Pia A. Johansson, German Camargo Ortega, Magdalena Götz
Institute for Stem Cell Research, Helmholtz-Center Munich, Munich, Germany

The size an organ is regulated by a tight balance between the self-renewal of stem cells and their generation of amplifying progenitors enlarging the progeny number. This is of particular relevance in brain development. To identify novel factors regulating neural stem cell maintenance versus differentiation we performed genome-wide expression analysis of neural stem cells at different developmental stages (Pinto et al., 2008, Mol Cell Neurosci.) and in the adult (Beckervordersandforth et al., 2010, Cell Stem Cell). We focused our attention of transcriptional regulators that were highly expressed in neurogenic neural stem cells (isolated from the embryonic forebrain, E14 radial glia, and the adult subependymal zone) and comparatively lower in neural stem cells undergoing gliogenesis. This approach yielded several candidates amongst them several AT-hook containing transcription factors, some also comprising the high mobility group. As expected from the arrays, expression of these factors was high in neural stem and progenitor cells in the developing forebrain, but largely absent in the adult brain except in stem cell niches. Towards functional analysis we used in utero electroporation of cDNA plasmids (Gain-of-function) and specific short hairpin RNA constructs (Loss-of-function). In particular, the AT-hook transcription factor lacking a HMG domain potently promoted neuronal differentiation. Already 1 day post electroporation, the proportion Ki67+, Pax6+ NSCs was significantly reduced, while the proportion of postmitotic cells was increased. Three days after electroporation these had differentiated largely into NeuN+ neurons. Conversely, knock-down experiments resulted in a decrease in differentiation as Ki67+ cells were increased, suggesting a vital role in embryonic neurogenesis. Genome-wide expression analysis performed shortly after overexpression or knock-down revealed exciting candidates that are presently investigated.

W-1134

IN VITRO CHARACTERIZED SALIVARY GLAND STEM CELLS RESCUE RADIATION-INDUCED HYPOSALIVATION

Lalitha S S. Nanduri[1], Mirjam Baanstra[1], Ronald P. van Os[2], **Robert P. Coppes**[1]
[1]Cell Biology and Radiation Oncology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands, [2]Department of Biology of Aging, European Research Institute for the Biology of Aging, University Medical Center Groningen, Groningen, Netherlands

Salivary glands are often exposed to radiation, during radiotherapy for head and neck cancers resulting in life-long salivary gland impairment, severely reducing the quality of life of the patients. Stem cell therapy may be an option to prevent radiation-induced hypo-salivation.
In mice, we recently showed(1) that transplantation of several types of stem cell marker expressing cells obtained from mouse salisphere are able to rescue radiation-induced hypo-salivation. The aim of this study is to identify the most potent stem cell of the mice salivary gland.
Murine salivary gland derived primary salispheres contain cells expressing CD24/CD29 that could be separated into four subsets; CD24hi/CD29lo, CD24hi/CD29hi, CD24 med/CD29hi and CD24lo/CD29hi cells. To test the self-renewal potential of the subsets 10,000 single cells/well were serial replated in matrigel to form secondary salispheres. Our results show that CD24hi/CD29hi, CD24 med/CD29hi cells have high secondary sphere forming potential with indefinite self-renewal for the CD24hi/CD29hi population. In our 3D matrigel-collagen differentiation matrix single cell derived spheres from all subsets form salivary organoid structures with the CD24lo or CD29lo group forming more lobular acinar like structures, whereas the CD24hi/CD29hi, and CD24med/CD29hi groups forming more ductal structures, suggesting that these populations contains more primitive stem/progenitor cells. Interestingly, all cells derived from spheres after more than 7 self-renewal passages are enriched for CD24hi/CD29hi. Moreover, when transplanted in locally 15 Gy irradiated salivary glands the success ratio of salisphere derived CD24hi/CD29hi cells (10000cells/mouse) in recovering gland function increased with passage number to 100% at passage 13, indicating selection of stem cells through culturing.

In conclusion, mice salisphere-derived cells that have a high expression of CD24 and CD29 have strong stem cell properties as they can self-renew, differentiate to salivary gland structures in vitro and superbly regenerate irradiation-damaged salivary glands in vivo. In vitro passaging of salivary gland cells further enriched this population for stem cells, indicating that this population most likely contains the stem cells of the salivary gland. Potentially this population could be used for stem cell therapy after radiotherapy for head and neck cancers.
Nanduri L.S.Y., Maimets M., Pringle S.A., van der Zwaag M., van Os R.P., Coppes R.P. Regeneration of irradiated salivary glands with stem cell marker expressing cells (2011) Radiotherapy and Oncology, 99 (3), pp. 367-372

W-1136

PLURIPOTENCY IN FALLOPIAN TUBE AND EPITHELIAL MESENCHYMAL TRANSITION

Farhana Ishrat Ghani[1], Mirjana Kessler[1], Thieck Oliver[1], Susan Jackisch[1], Christina Fotopouolu[2], Thomas F. Meyer[1]
[1]Molecula Biology, Max Planck Institute for Infection Biology, Berlin, Germany, [2]Department of Gynocology, Charite University Medicine, Campus Virchow, Berlin, Germany

Oxidative stress response, inflammation and regeneration during the reproductive years argue for the existence of pluripotent mechanisms in fallopian tube mucosa to facilitate these extensive changes. Also implication of fallopian tube in etiology of ovarian cancer might indicate the existence of stem cells in this organ. We identify stem cell potential within fallopian tube epithelium and establish long term culture in vitro. Monolayer culture was obtained by cultivating mucosal folds in 2D culture and 3D organoid culture system could be established in Matrigel by providing optimal growth conditions with paracrine signaling components. Differentiated spheres, exhibiting all markers of mature, polarized, fallopian tube epithelium, could be propagated in vitro for longer than 4 months, supporting the presence of stem cells as the “engine” of proliferation potential. Active proliferation regions, resembling mucosal folds in tissue, were confirmed by immunohystochemistry. In effort to identify subpopulation of epithelial cells with stem cell capacity we show that sorted Epcam high cells have the highest organoid formation potential in 3D culture by limited dilution organoid culture assay. Other stem cell markers like alkaline phosphatase, Lgr5, CD133 were also expressed in these cells. Furthermore, 2D cultured cells undergo epithelial mesenchymal transition which is the most important event during organogenesis and cancer metastasis. In early passages, epithelial marker E-cadherin is highly expressed and in late passages EMT marker Snail and mesenchymal marker N-cadherin are strongly expressed and cells show mesenchymal morphology. Therefore, fallopian tube is a nice model to study stem cell characteristics and EMT of epithelial cells.

W-1137

CHARACTERIZING PUTATIVE THYMIC EPITHELIAL PROGENITOR CELLS IN THE ADULT MURINE THYMUS

Kahlia Y.P Wong[1], Natalie L. Seach[1], Maree V. Hammett[1], Marco Barsanti[1], Daniel Gray[2], Richard Boyd[1], Ann Chidgey[1]
[1]Monash Immunology and Stem Cell Laboratories, Monash University, Melbourne, Australia, [2]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

The thymus is the primary source of T cells for adaptive immunity, in which thymic epithelial cells (TECs) play a crucial role. Thymic function and naïve T cell output is optimum during the early years of life but progressively declines, resulting in <10% of functional tissue remaining by midlife. The decrease in naïve T cell output leads to compromised immunity in the elderly and is further exacerbated by cytoablative treatments, such as irradiation and chemotherapy in bone marrow transplant and cancer patients.
Strategies to regenerate the thymic microenvironment include sex steroid ablation and growth factor transfusions. However, from a clinical perspective, optimal recovery of the naïve T cell pool remains a challenge. An alternative approach may be to activate resident TEC progenitors/stem cells within the adult thymic microenvironment to mediate internal thymic regrowth and thus, T cell output.

Whilst evidence supports the derivation of cortical (cTEC) and medullary (mTEC) epithelial lineages from a common bipotent progenitor in the postnatal thymus, the identity of these cells remains elusive. Herein we characterize the adult thymic epithelium and demonstrate that progenitor capacity is retained exclusively by a subset of cortical TECs expressing high levels of Sca-1 and low levels of MHC Class II ($cTEC^{lo}$). Using label retention studies, this subset was found to retain BrdU at 6 months post chase and was preferentially localized to the cortico-medullary junction of adult thymic tissue. Adult epithelium grows poorly in 2D culture however, when placed in a 3D Matrigel co-culture system, $cTEC^{lo}$ efficiently formed colonies and repeatedly generated medullary lineage following serial purification and passaging *in vitro*. Furthermore, when combined with embryonic stroma and engrafted under the kidney capsule, reaggregates containing putative cTEC progenitors generated pockets of thymic tissue containing cells of both medullary and cortical lineage *in vivo*. These cells persisted at 12 weeks post-engraftment however long-term serial grafting will be required to assess the self-renewal potential of the $cTEC^{lo}$ subset.

In summary, these findings suggest that progenitor, and possibly stem cell, potential is retained in the $cTEC^{lo}$ subset in postnatal thymic tissue. Further studies will help refine the molecular pathways involved in thymic stem or progenitor cell activation, and potentially contribute to strategies for endogenous repair of thymus function.

Embryonic Stem Cell Clinical Application

W-1142

TRANSPLANTATION OF XENO-FREE, SORTED HUMAN EMBRYONIC-DERIVED NEURAL STEM CELLS INTO THE INTACT AND TRAUMATICALLY INJURED RODENT BRAIN

Daniel L. Haus[1], Eric Gold[1], Harvey Perez[2], Hal X. Nguyen[2], Aileen J. Anderson[3], Brian J. Cummings[3]

[A]Anatomy and Neurobiology, [B]Sue and Bill Gross Stem Cell Center, [C]Physical Medicine and Rehabilitation, [1]University of California, Irvine, Irvine, CA, United States

Traumatic brain injury (TBI) is one of the leading causes of death and disability worldwide, with an estimated 1.7 million individuals affected each year in the United States alone. Limited treatment options exist, which include stabilizing patients after the initial traumatic event as well as providing limited physical and cognitive rehabilitation. However, therapies to facilitate cellular repair and treat the underlying source of TBI-related deficits have remained elusive. Transplantation of human neural stem cells has the potential to afford such repair via multiple mechanisms, including cellular replacement and/or neurotrophic support. Currently, multiple cell transplantation-based clinical trials are on-going for conditions such as amyotrophic lateral sclerosis (ALS) and spinal cord injury. However, human neural stem cell transplantation has not yet been fully evaluated long-term in an appropriate animal model of TBI. Here we report results from the development and subsequent transplantation of Xeno-Free (XF) human embryonic stem cell-derived neural stem cells (hES-NSCs) into the intact and traumatically injured immunodeficient rodent brain. Shef4 and Shef6 XF hES-NSCs expressed markers characteristic of neural stem/progenitor cells in vitro via immunocytochemistry (ICC) and qPCR (e.g. Nestin, Sox2) and retained a normal karyotype. Magnetic sorting (MACS, Miltenyi Biotec) was used to further enrich XF hES-NSCs for a self-renewing stem cell population (CD133+) and remove potential contaminating CD34+ cells of the hematopoietic and endothelial lineages. Multipotency of differentiated CD133+/CD34- Shef6 XF hES-NSCs was confirmed at 28 days in vitro via expression of neural lineage markers (e.g. ß-TubIII, GFAP, and Olig2). Additionally, unsorted and CD133+/CD34- sorted Shef6 XF hES-NSCs differed in cellular morphologies and growth dynamics in vitro, with unsorted cells having increased cytoplasmic volume and a slower rate of expansion. Upon stereotaxic transplantation into the intact immunodeficient NOD-Scid mouse hippocampus, unsorted Shef6 XF hES-NSCs failed to engraft (0/7, 0%) when examined 12 weeks post-transplantation. Conversely, CD133+/CD34- sorted Shef6 XF hES-NSCs engrafted in the intact hippocampus with a high rate of success (10/10, 100%), with a majority of SC121 positive human cells expressing the neuronal marker ß-TubIII at 12 weeks post-transplantation. When transplanted into the traumatically injured Athymic Nude (ATN) rat brain 9 days after a controlled cortical impact (TBI-0310, Precision Systems and Instrumentation, LLC), CD133+/CD34- Shef6 XF hES-NSCs engrafted, migrated, and extended long processes around the injury penumbra as well as into the contralateral hemisphere via the corpus callosum. Human cell projections were found spanning more than

3mm anterior to posterior and 5mm contralateral from the initial injection sites at 8 weeks post-transplantation. In summary, our preliminary data has demonstrated that sorting (CD133+/CD34-) of Shef6 XF hES-NSCs improves cell engraftment and migration in a sub-acute TBI model, and thereby may produce a more clinically translatable cell population. Future studies will aim to further explore contralateral cell migration/process extension and assess the efficacy of cellular transplantation in promoting behavioral recovery after TBI.

W-1143

HUMAN DEVELOPMENTAL CHONDROGENESIS AS A BASIS FOR THE ENGINEERING OF CHONDROCYTES FROM PLURIPOTENT STEM CELLS.

Ling Wu, Brooke Latour, Stephanie Gonzalez, Saumya Shah, Frank Petrigliano, David R. McAllister, **Denis Evseenko**
UCLA, Los Angeles, CA, United States

Articular cartilage is a highly specialized tissue formed from chondrocytes that protects the bones of diarthrodial joints from forces associated with load bearing and impact, and allows nearly frictionless motion between the articular surfaces. Cartilage injury and lack of cartilage regeneration often lead to osteoarthritis currently affecting more than 25 million people in the United States alone, making joint surface restoration a major priority in modern medicine. None of the previously published studies have reported the generation of a highly purified population of pluripotnet stem cells (PSC)-derived chondrocytes, instead demonstrating the generation of a likely heterogeneous population capable of depositing chondrogenic matrix molecules, the cell of origin of which cannot be tracked in these systems. As a result, immunophenotypes and functional characteristics of the earliest cartilage cells derived during differentiation of PSC were not known. To the large extend this knowledge was missing due to the complete lack of the developmental studies dissecting the earliest stages of human chondrogenesis. In this study we have isolated and characterized, for the first time, pre-chondrocytes and chondrocytes at 6-17 weeks of human development. Pre-chondrocytes represent a unique, transitional population of cartilage-committed cells at the earliest stages of articular joint development that gives rise to joint forming definitive peri-articular chondrocytes. Genome wide comparison of pre-chondrocytes isolated at 6 weeks of development with peri-articular chondrocytes in fully formed joints at 17 weeks of human development led to the identification of several markers for immunophenotypic tracing and FACS isolation of both cartilage cell types. The identification of specific molecular signature for the earliest pre-chondrocytes and peri-articular chondrocytes will now permit further delineation of the molecular regulation of the first stages of chondrogenic commitment in humans. It may also contribute to understanding of how these processes are affected during aberrant chondrogenesis in disease states. Finally, characterization of the earliest primary cartilage cells provides essential knowledge for the generation of highly purified chondrocytes from PSC and representing a unique target for cartilage tissue engineering.

W-1144

MATURATION OF INDUCED NEURONAL CELLS GENERATED FROM ADULT AND POSTNATAL FIBROBLASTS FACILITATED BY SMALL MOLECULES

Cheen Euong Ang, Marius Wernig
Bioengineering, Stanford University, Stanford, CA, United States

Since our lab reported the first direct conversion of mouse and human fibroblasts to induced excitatory neuronal (iN) cells, several groups have since reported using the same four transcription factors (Brn2, Ascl1, Myt1l and NeuroD1) or in combination with other transcription factors or microRNAs to generate other neuronal subtypes. While mouse embryonic fibroblast to iN cell conversion is generally efficient and yields synaptically mature iN cells, the reprogramming efficiency of human postnatal or adult fibroblasts to iN cells hovers around 2% and most resulting neurons are of very immature appearance. Herein, I will describe our efforts in employing a high throughput screening strategy to find small molecule inhibitors or agonists which can improve iN reprogramming efficiency. To date, an inhibitor of the BMP and an agonist of the PKC pathway were found to be helpful in increasing the efficiency of iN reprogramming by up to 2.5 fold for a mere three day drug treatment. Further characterizations using

immunofluorescence showed that the induced neuronal cells are positive for multiple mature neuronal markers as early as 2 to 2.5 weeks post doxycycline induction which is faster than using BAMN alone. These results provide (a) a basis on which further screenings can be done to find other small molecules that act synergistically with the two small molecules and (b) a protocol in which induced neurons can be generated in sufficiently high amount for human disease modelling and drug screening purposes.

W-1145

THE APPLICATION OF MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) AS ROUTINE EXAMINATION METHOD FOR GENETIC STABILITY **OF HUMAN EMBRYONIC STEM CELLS**

Jeoung Eun Lee[1], Sung Han Shim[2], Myung Sun Shim[1], Yuri Kim[1], Sung-Geum Lee[1], Dong Ryul Lee[1]
[1]CHA Stem Cell Institute, CHA Uiversity, Seoul, Korea, Republic of, [2]Dept of Biomedical Science, CHA Uiversity, Seoul, Korea, Republic of

Human Embryonic Stem Cells (hESCs) offer a renewable source for cell based therapy and regenerative medicine by their self-renewal ability and differentiation ability to all kinds of cell type in the body. For clinical application, genetic stability of hESCs should be proven during cultivation and differentiation in the chromosomal level at least. In several studies regarding the chromosomal integrity during long term culture, the most results showed that hESCs were stably maintained after freezing/thawing and long-term culture. But some reported that hESCs had developed chromosomal aberration during in vitro culture. Therefore, hESCs have to be routinely tested at least chromosomal level to verify deleterious changes during maintenance and differentiation. The MLPA is a fast method designed for simultaneous quantification of copy numbers of several dozens of specific genomic sequences. MLPA is easy to perform and requires only 20ng of DNA sample, and is mainly used for diagnosis such as copy number changes of human genomic DNA sequences. Here, we reported the results of genetic stability test by MLPA (SALSA MLPA Kit P070; detection of deletion/duplication) and traditional karyotyping using G-banding in cultivated 10 hESC lines. In early and late passages (cultured for more than 50 passages), chromosomal abnormality of CHA-hES 15, 20, 31, 32, 36, 45, B3 (maintained on mouse feeder cells), 40, 42 (maintained on human feeder cells in xeno-free culture condition), and H1 (maintained on mouse feeder cells) was analyzed by using those two methods. In the present study, the MLPA results corresponded with the karyotyping results. CHA-hES 15, 20, 31, 40, B3 and H1 were 46,XY, and CHA-hES 36 was 46,XX. CHA-hES 32 was 46,XX,inv(9). Interestingly, CHA-hES 42 and 45 were chromosomally abnormal at early passage, 47,XY,+5 and 47,XX,+16 respectively, and then changed spontaneously to normal karyotype during cultivation, 46,XY and 46,XX respectively. It was suggested that the MLPA does not detect polyploidy (like triploid/tetraploid) and translocation, but can detect deletion or duplication of chromosome easily and quickly. Therefore, we may suggest that MLPA analysis can be applicable for routine chromosome analysis of hESCs due to their convenience after MLPA and karyotyping were performed at early stage. Also, when MLPA detected the deletion or duplication, karyotyping should be performed for confirming the genetic abnormality of hESCs.

W-1146

INEFFICIENT INDUCTION OF HUMAN EMBRYONIC STEM CELLS TO KERATINOCYTE STEM CELLS POPULATION DURING EARLY STAGE OF EPIDERMAL COMMITMENT: A KEY PLAYER IN LIMITED PROPAGABILITY?

Mehdi Mohammad Movahednia, Fahad Karim Kidwai, Yu Zou, Huei Jinn Tong, Tong Cao
Dentistry, National University of Singapore, Singapore, Singapore

Background: Upon first derivation of human embryonic stem cells (hESCs), they attracted lots of excitements, concentrated in developmental research models and future regenerative therapies, due to their unique ability to differentiate into all 3 germ layers *in vitro*. Successful hESCs differentiation toward particulate lineage can be achieved through exposure to selective growth factors and/or by providing a co-culture system with specific inducing lineage. In this notion various differentiation methods toward hESCs-derived keratinocyte (hESCs-Ker) have been reported to provide a pure epidermal population with extended replicative capacity. However, in all of those methods presence of xenogeneic compounds was far from clinically accepted procedures. Recently, we reported the successful

establishment of Autogenic Feeder Free (AFF) system provided by extra cellular matrix (ECM) derived from hESCs-fibroblast, able to provide pure population of hESCs-derived keratinocyte (AFF-Ker) in xeno free environment after 10 days differentiation. However, research and future clinical applications of AFF-Ker system were hampered by their limited propagation (till day 40 day). Therefore, it was our interest to unravel this limited replicative capacity and early senescence in compare to hESCs-Ker, differentiated in presence of feeder. We identified hierarchy of keratinocyte population during differentiation and found that keratinocyte stem cells (KSCs) are the key regulatory factor in hESCs-Ker growth capacity. We speculated that alteration in culture condition during differentiation can increase this population and consequently extends hESCs-Ker propagation.
Methods: hESCs-Ker was provided in two differentiation system, autogenic co-culture (ACC) system in which hESCs cultured on hESCs-fibroblast and autogenic feeder free (AFF) system, utilizing hESCs-fibroblast ECM as a microenvironment. Culture condition alteration performed during 10 days of differentiation in AFF system following by KSCs population quantification by flow cytometry analysis.
Results: Efficient induction to KSCs at early stage of epidermal commitment plays a critical role in hESCs-Ker future growth capacity**.** hESCs-Ker in AFF system posed limited replicative capacity due to the low percentage of KSCs population. Alternatively, KSCs population was found to be significantly higher in propagable hESCs-Ker, in ACC system. Induction to pure population of KSCs in AFF system was performed through culture modification during epidermal commitment. AFF-Ker differentiated in modified condition showed 16±2.4 (SE) population doublings over 70 days with delayed expression of senescence associated β-galactosidase (SA-β-Gal) activity. Moreover, AFF-Ker with extended propagation showed decreased accumulation of cyclin-dependent kinase inhibitor proteins, p16 and p21, concomitant with slower telomere erosion rate.
Conclusion: hESCs-Ker with extended culturing time provided by efficient induction of KSCs at early stage of epidermal commitment. These findings open a new perspective in extending hESCs-derived keratinocyte as an authentic research model. Moreover, this study will enhance the industrial and future clinical application of hESCs-derived keratinocyte.

W-1147

BETA-CATENIN SIGNALING PATHWAY REGULATES THE NON-TUMORIGENESIS OF HUMAN EMBRYONIC STEM CELLS CULTURED ON HUMAN UMBILICAL CORD STROMAL CELL FEEDERS

Dah-Ching Ding[1], Yu-Hsun Chang[2], Tang-Yuan Chu[3]
[1]Dept of Obstetrics and Gynecology, Buddhist Tzu Chi General Hospital, Tzu Chi University, Hualien, Taiwan, [A]Dept of Pediatrics, [B]Dept of Obstetrics and Gynecology, [2]Buddhist Tzu Chi General Hospital, Hualien, Taiwan

The expansion of pluripotent human embryonic stem cells (hESCs) requires a culture on feeder layers of mouse embryonic fibroblasts (MEF). This culture model often involves immunogenic contamination such as xeno-carbohydrate, and inevitably forms teratoma in vivo. We used human umbilical cord-derived mesenchymal stem cells (HUCMSC) as the feeder for hESCs. Under a mitomycin-inhibited HUCMSC feeder, the hESCs maintained the features of embryonic stem cells (pluripotency, immortality, unlimited undifferentiated proliferation, and maintenance of normal karyotypes) after a prolonged culture of more than 20 passages.
Notably, in extensive trials, no teratomas were formed in xenografts in NOD/SCID mice, however subsequent resumption of teratoma formation was noted upon transient co-culturing with MEF. HUCMSC feeders may confer an “epiblast-like” status on hESCs. Interestingly, among the four pluripotency-conferring genes, MYC was found to be down-regulated in hESCs co-cultured with HUCMSC. We propose that MYC-mediated tumorigenic pathways are down-regulated in hESCs under this coculture model. This means that hESCs on HUCMSC may be reprogrammed to a non-tumorigenic epiblast such as stem cells.
In this study, we found that the β-catenin signaling pathway is an important signaling pathway which regulates the tumorigenicity of hESCs. We demonstrated that the β-catenin antagonist, FH535, could down-regulate β-catenin and c-MYC transcription and translation in vitro. Furthermore, FH535 also reduced the teratoma formation rate of hESCs/MEF in vivo. We also demonstrated that the β-catenin enhancer, LiCl, could up-regulate β-catenin and c-MYC

transcription and translation in vitro. Moreover, LiCl could promote the teratoma formation of hESCs/HUCMSC in vivo.
Based on these findings, we conclude that the WNT/β-catenin pathway is an important but not the only signaling pathway which controls the tumorigenicity of hESCs.

Hematopoietic Cells

W-1151

ANGIOPOIETIN-LIKE PROTEIN RECEPTORS STIMULATE HSC DEVELOPMENT THROUGH DIRECT INTERACTION WITH NOTCH.

Michelle I. Lin[1], Emily N. Price[1], Sruthi Satishchandran[1], Charles W. Carspecken[1], Audrey Uong[1], Anthony DiBiase[1], Jon C. Aster[2], Richard M. White[3], Leonard I. Zon[1]

[1]Harvard Medical School, HHMI, HSCI, Hematology/Oncology, Children's Hospital Boston, Boston, MA, United States, [2]Pathology, Brigham and Women's Hospital, Boston, MA, United States, [3]Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, United States

Members of the angiopoietin-like protein (angptl) family have recently been identified as novel growth factors, capable of stimulating ex vivo expansion of mouse and human HSCs. However, their requirement during development and their mechanism of action are still very much unknown. To first recapitulate the effects of exogenous angptl on HSC expansion, we generated a stable heatshock inducible transgenic zebrafish line to overexpresses angptl2 and found that these embryos showed a significant increase in cmyb- and runx1-positive HSCs in the aorta-gonad-mesonephros (AGM) region, the site of definitive hematopoiesis. Conversely, anti-sense morpholino knockdown of angptl1 and 2 resulted in a decrease in cmyb/runx1-positive HSCs in the AGM, suggesting that angptls are sufficient and required for definitive hematopoiesis. These double morphants also displayed a disruption in vascular development and differentiation prior to the defect observed in the AGM, indicating that angptl regulation of HSC development occurs through an early specification of the hemogenic endothelium. Because angptl morphants phenotypes resemble that found in the notch mutant mindbomb, mib, we asked if there is a genetic interaction between notch and angptl signaling. Using a transgenic notch reporter line, we see that knocking down angptl1 and 2 caused decreased notch signaling. Surprisingly, the HSC defects normally observed in mib embryos were rescued when angptl2 is overexpressed, implying that angptl2 acts downstream of mindbomb signaling. When we overexpressed a constitutively active form of notch in angptl1 and 2 double morphants, restored HSC formation was observed. Together, these genetic data corroborated our hypothesis that angptls can regulate notch signaling. To further dissect this relationship, we stimulated cultured human CD34+ cells with purified Angptl2 and found an increase in Notch receptor cleavage, indicating that Angptl2 can induce Notch receptor activation. Through endogenous co-immunoprecipitation experiments in CD34+ cells, we also found that the Angptl receptor, LILRB2, interacted with Notch receptor either in the transmembrane or intracellular region. This strongly indicates a direct regulation of Notch activation/signaling by Angptls through physical interactions between Notch and Angptl receptors. Finally, in order to examine downstream signaling of Angptl-mediated Notch activation, we performed ChIP-seq for Notch in Angptl2-stimulated CD34+ cells and found enrichment for Myc binding elements. Independent microarrays performed from the same cells also revealed a strong Myc signature through GSEA analysis. Thus, to test the hypothesis that myc is a common downstream mediator for angptl and notch signaling, we overexpressed zebrafish myc-b in angptl1 and 2 double morphants or mib mutants, and found a significant rescue in HSCs formation in the AGM. Together, these results indicate that angptl can regulate notch signaling through receptor interaction, leading to activation of myc target genes during definitive hematopoiesis. Our data provide new insights to the previously uncharacterized angptl signaling during HSC development and present a novel mechanism of action for non-canonical notch activation.

W-1152

HEMATOPOIETIC ENGRAFTMENT OF AMNIOTIC FLUID STEM CELLS FOLLOWING IN UTERO TRANSPLANTATION

Stavros P. Loukogeorgakis[1], Panicos Shangaris[2], Enrica Bertin[4], Panagiotis Maghsoudlou[1], Michael Blundell[3], Luca Urbani[1], Valentina Spinelli[1], Durrgah Ramachandra[1], Adrian Thrasher[3], Michela Pozzobon[4], Anna L. David[2], Paolo De Coppi[1], Paolo De Coppi[1]

[A]Surgery Unit, ICH, [B]Institute for Women's Health, [C]Center for Immunodeficiency, ICH, [1]University College London, London, United Kingdom, [2]Regenerative Medicine Laboratory, University of Padua, Padua, Italy

Background:
Fetal immune tolerance is the most compelling rationale for prenatal stem cell therapy for congenital blood disorders. However, experimental in utero transplantation (IUT) of allogenic hematopoietic stem cells gives limited chimerism in the blood, and maternal humoral and cellular immune responses are thought to be to be responsible for loss of engraftment. Amniotic fluid stem cells (AFSC) are an appealing cell source for IUT as they are autologous to the fetus, non-immunogenic to the mother, and have shown hematopoietic potential when transplanted postnatally. The aim of the present study was to determine whether IUT of autologous (congenic) AFSC would result in hematopoietic engraftment in immune-competent mice.

Methods:
IUT was performed in timed-pregnant wild-type C57BL/6J mice (n=3; CD45.2+). To mimic autologous AFSC IUT, lineage-depleted/c-Kit+ AFSC were isolated at E13.5 from C57BL/6J dams expressing CD45.1 (n=2; CD45.1+; congenic AFSC). IUT was performed at E13.5, and 10,000 freshly isolated AFSC were injected into the peritoneal cavity of each fetus (n=15). Donor cell engraftment in transplanted pups was assessed at 4 weeks of age by flow cytometry as the percentage of CD45.1+ cells isolated from blood and hematopoietic organs. Chimerism was defined as more than 1% (of total) CD45.1+ cells. Bone marrow (BM) derived cells from chimeric pups were used to perform colony-forming assays. Results are expressed as mean±SE.

Results:
Freshly isolated c-Kit+ AFSC expressed pluripotency (c-myc, Klf4, Oct4, Sox2, SSEA1), as well as hematopoietic markers (Sca1, CD34, CD45.1). The miscarriage rate following IUT was 33.3% (5/15) and there were no maternal complications. Chimerism in the blood and BM was observed in 100% (4/4) of transplanted pups at 4 weeks (CD45.1+ cells in blood: 5.8±1.2% of total; BM: 10.9±1.6% of total). Transplanted (CD45.1+) cells contributed significantly to hematopoietic stem cell (7.0±1.3% of CD34+; 7.2±1.9% of c-Kit+) and lymphocyte (8.8±2.2% of CD4+ cells; 11.6±2.0% of CD8+) populations in the blood, and similar results were observed in BM (18.1±1.8% of CD34+; 15.2±0.8% of c-Kit+; 19.4±1.7% of CD4+; 12.7±1.2% of CD8+). Moreover, there was substantial engraftment in other haematopoietic organs including the thymus (CD45.1+ cells: 10.4±5.3% of total; 38.5±6.8% of CD34+; 39.3±5.3% of c-Kit+; 38.8±5.4% of CD4+; 41.5±2.3% of CD8+), spleen (5.2±1.0% of total) and liver (4.3±0.3% of total). Colony forming assays of BM cells from chimeric pups (n=2) demonstrated formation of hematopoietic colonies (BFU-E: n=18; CFU-GM: n=72; CFU-M: n=15), and CD45.1+ cells represented 6.2±1.2% of the total cells harvested after 12 days of culture.

Conclusion:
Prenatal transplantation of AFSC results in successful hematopoietic engraftment in an immune-competent murine model of autologous (congenic) stem cell IUT. Further work will determine whether these engrafted cells have hematopoietic potential when transplanted secondarily in immune-deficient mice and whether IUT of AFSC is effective for prenatal therapy in murine models of congenital blood disorders.

W-1153

REGULATORY FUNCTION OF HEMATOPOIETIC STEM/PROGENITOR CELLS THROUGH THE ARYL HYDROCARBON RECEPTOR

Yoko Hirabayashi[1], Byung-Il Yoon[2], Isao Tsuboi[3], Jun Kanno[1], Yoshiaki Fujii-Kuriyama[4], Tohru Inoue[3]

[1]Division of Cellular and Molecular Toxicology, Center for Biological Safety and Research, National Institute of Health Sciences, Tokyo, Japan, [2]Laboratory of Histology and Molecular Pathogenesis, College of Veterinary Medicine, Kangwon National University, Chuncheon, Korea, Republic of, [3]Department of Function and Structural Medi-

cine, Nihon University School of Medicine, Tokyo, Japan, [4]Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan

To elucidate the biological functions of the aryl hydrocarbon receptor (AhR), which transmits various xenobiotic responses, it may be strategically worthwhile to analyze tissue stem cells as a model because the expression level of AhR tends to gradually decrease as stem cells differentiate. Because we have recently found that AhR-knockout (KO) mice show an earlier onset of spontaneous neoplasms and a much shorter lifespan than wild-type mice, AhR is assumed to have a suppressor gene function. In this study, we focus on the biological function of AhR in hematopoietic progenitor cells and the cell cycle regulation of these cells to elucidate the relevant mechanism that may explain such an advantageous function of AhR. In the comparison of AhR-KO mice with wild-type mice, 1) the cell cycle status of hematopoietic stem/progenitor cells (colony forming unit (CFU) granulocyte-macrophage (GM); day 9 CFU in spleen (CFU-S9); and day 13 CFU-S (CFU-S13)) was examined by evaluating cycling stem/progenitor cells with continuous incorporation of bromodeoxyuridine (BrdUrd) *in vivo* for up to 13 weeks followed by exposure to ultra-violet A light to eliminate only BrdUrd-incorporating cells, and 2) comprehensive gene expression analysis of bone marrow cells in the steady state was carried out using an Affymetrix GeneChip® Mouse Genome 430 2.0 Array. As a result, 1) AhR-KO mice showed an increased percent kill (percentage of BrdUrd-incorporating stem/progenitor cells) of CFU-S13 or a decreased percent kill of CFU-GM during the initial 1 hr (S phase fraction); a shortened doubling time of CFU-S9: and an increased percent kill of all the stem/progenitor cells, CFU-GM, -S9, and -S13, at a plateau level (entire cycling fraction). In other words, although those from AhR-KO mice showed different times to reach the plateau level, none of them showed continuously increasing BrdUrd incorporation rate, but maintained the level at least until 13 weeks, as in the wild-type mice, which once reached a plateau level after 8 weeks of age then kept the level until 18 months of age. 2) Three hundred and fifteen probe sets were selected as showing significantly different expression levels between AhR-KO and wild-type mice. Among them, 276 genes including 51 cell-cycle regulators were identified by Ingenuity Pathway Analysis. Moreover, their biological functions, which were considered on the basis of their expression levels, were elucidated as to be related to the acceleration of cell cycle (e.g., up-regulation of bromodomain-containing protein 4 and pim-1, down-regulation of transcription factor 3), suppression of apoptosis (e.g., up-regulation of BCL2-like 1, down-regulation of caspase 7 and Fyn), and tumorigenesis (e.g., down-regulation of mitogen-activated protein kinase 9 /Jun kinase 2, Smad4, and RNA polymerase II, TATA box binding protein-associated factor). Thus, steady-state hematopoiesis is presumed to be suppressed via AhR signaling by a possible physiological ligand, which is as yet not identified in wild-type $AhR^{+/+}$ mice. The possible regulation of hematopoietic stem cells that maintains the fraction of dormant hematopoietic stem cells may have mechanistic relevance in terms of a possible genomic stabilization by AhR, which should be elucidated. Nevertheless, such a deceleration of the cell cycle in hematopoietic stem/progenitor cells contributes to a longer stem cell survival, which may be the reason for the extension of animal lifespan.

W-1154

ACCELERATIVE EFFECT OF AUTOLOGOUS BONE MARROW ON HEALING OF MANDIBULAR FRACTURES IN MALE DROMEDARY CAMELS

Fahd Al-sobayil[1], FA Ahmed[1], MA Al-hawas[1], NA Aljarbou[1], TM Bakheet[2], Abdullah O. Alawad[3], Mohammed A. Al-darweesh[4], **Saleh Altuwaijri**[4]

[1]Qassim Uiversity, Qassim, Saudi Arabia, [2]Maternity and children hospital, Ministry of Health, Jeddah, Saudi Arabia, [3]King Abdulaziz City for Science & Technology, Riyadh, Saudi Arabia, [4]SAAD Research & Development Center ,SAAD Specialist Hosptial, Alkhober, Saudi Arabia

Recent researches have shown that mandibular fractures are the most common fractures in male camels. Healing of mandibular fracture in camels takes relatively long time in which the animal might die due to its inability to eat. Bone marrow contains many cellular elements that may contribute to fracture repair. Several studies on laboratory animals and humans have shown that injection of bone marrow cells significantly enhances healing of bone fracture. The aim of this study was to investigate the effect of bone marrow cells on the acceleration of mandibular fracture healing in male dromedary camels. In addition to physical examination, bone healing was evaluated by bone biomarkers (formation and resorption) and x-ray imaging techniques. Twenty four mandible-fractured adult male camels were divided into four groups:6 were fixed with interdental wiring technique with injecting bone marrow sample at the fracture line (IWBM), 6 were fixed with interdental wiring without adding bone marrow(IW), 6 were fixed with screws and plates with injecting bone marrow at the fracture line (SPBM), and 6 were fixed with only screws and plates (SP). Autologus bone marrow samples were collected from the tuber coxae (6 Ml) and directly injected at the fracture line on the day of bone fixation surgery (day 0) and 14 days post surgery (day 14). Blood samples were collected weekly and x-ray radiography were performed every 14 days. Bone formation biomarkers including osteocalcin (OC) and bone alkaline phsphatase(BAP) and bone resorption biomarkers including pyridinoline (PYD) and deoxypyridinoline (DPY) crosslinks were determined in the serum samples. The mean levels of calcium and phosphorus were also determined in the serum samples. The time took up for complete fracture healing was significantly shorter in groups using bone marrow compared with those without injecting BM. The levels of OC and BAP were significantly increased in all groups, but they were higher starting at day 14 post surgery in groups with BM compared with those without BM. A positive correlation was observed between x-ray radiographs and the concentration of bone formation biomarkers starting from day 28 post surgery. It was concluded that injecting fresh bone marrow directly on the fracture line was able to accelerate the healing of mandibular fracture in dromedary camels.

W-1155

REPROGRAMMING HUMAN COMMITTED ERYTHROBLASTS EXPANDED FROM BLOOD TO MULTI-POTENT BLOOD PROGENITORS

Bin-Kuan Chou, Xiaosong Huang, Siddharth Shah, Linzhao Cheng
Institute for Cell Engineering, Johns Hopkins Univ, Baltimore, MD, United States

Despite extensive efforts in the past decades to expand transplantable CD34+ human hematopoietic stem/progenitor cells (HSPCs) that are able to give rise to multiple lineages of human hematopoietic cells after clinical transplantation, more research is still required to achieve ex vivo expansion of human HSPCs. Ex vivo production of transplantable HSPCs from human ES cells and more recently from iPS cells has been actively pursued in the past several years, but so far this approach has proved difficult. This is likely due to the lack of a culture condition enabling self-renewal and/or anti-differentiation of human HSPCs in culture. The concept of cellular fate conversion by the iPS reprogramming factors that convert cells to a self-renewing state may provide a novel route to achieve HSPC expansion. We took a different approach by reprogramming human committed blood mononuclear cells (MNCs) back to HSPCs. To this end, we started with cord blood MNCs that have been cultured under an erythroblast culture condition for 7-10 days. As predicted, the expanded cells are mostly erythroblasts expressing CD36 and CD235a,

able to form erythroid colonies (very few myeloid colonies), and lacking lymphoid and myeloid cells as we previously reported (Chou et al., 2011). We further purified the starting cells by sorting the CD34-CD36+CD45+ cell population, in order to exclude possible HSPC (CD34+) contamination. The sorted cells were infected by a single retroviral vector expressing the standard 4 iPS factors. Instead of putting cells under a culture condition of iPS cell induction, the infected erythroblasts at day 2 post transduction were cultured under conditions favoring HSPC growth that include SCF, TPO and FLT3 ligand. In the absence of feeder cells, we observed a transient population of CD34+CD45+ cells characteristic of human HSPCs. Similar to the culture of primary CD34+ HSPCs, these induced CD34+CD45+ cells could not be sustained for more than 3 weeks: resulting cells are either CD34-CD45+ cells in suspension or CD34+CD45- adherent cells. In the presence of stromal cells, however, we observed the proliferation of CD34+CD45+ cells from day 14 post-infection that continued for at least 8 weeks. The standard methylcellulose colony-forming assays confirmed the CD34+CD45+ cells can form both myeloid and erythroid colonies (1% and 0.2%, respectively). The conversion from human CD34-CD36+ committed erythroblasts to CD34+ HSPCs within 2-3 weeks required the iPS reprogramming vector; a GFP control vector is insufficient. Our finding suggests that human committed blood cells can be reprogrammed back to multipotent and expandable blood progenitors, without the need of iPS cell derivation and expansion. We are currently testing the potential of the directly reprogrammed and expanded CD34+CD45+ cells to lymphoid lineages and to engraft in mouse models. We are also examining if we can achieve the same result by using non-integrating vectors and adult MNCs as we did previously for iPS cell derivation (Chou et al., 2011). If successful, our approach provides an alternative method to expand patient-specific HSPCs ex vivo for research and for future clinical transplantation.

W-1156

A ROLE FOR STROMA-DERIVED OSTEOPONTIN IN HEMATOPOIETIC STEM CELL AGING

Novella Guidi[1], Karin Doerr[1], Maria Carolina Florian[1], Hartmut Geiger[2]

[1]Dermatology and Allergic diseases, Ulm University, Ulm, Germany, [2]Experimental Hematology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States

Hematopoietic stem cells (HSCs) are located within bone marrow (BM) in a specific microenvironment referred to as the stem cell niche. HSCs as well as more differentiated hematopoietic cells interact in the BM with non-hematopoietic cells (CD45- cells) called stroma cells. The interaction of stroma cells with HSCs is central to hematopoiesis for regulating cell proliferation, self-renewal, differentiation and location. This interaction is supposed to be regulated by cytokines as well as signalling mechanisms that are at least in part initiated by cell-to-cell interactions. Aging of HSCs is linked to a decreased immune response, an increase in myeloid disease and a reduced regenerative capacity. It is supposed to be driven by primarily stem cell intrinsic mechanisms, and only more recently also stem cell extrinsic mechanisms of HSC aging are being discovered. We thus hypothesized that HSC niches age, and that these aging-associated changes impair HSCs function.
Flow cytometric analyses of BM stroma cells located at the endosteum from either young (2 month) or old C57BL/6 mice (22 month) demonstrated a significant decrease in the frequency of CD45- cells and of the osteoblastic subpopulation CD45-CD44+ in aged mice. Osteopontin (OPN) is a secreted glycoprotein expressed by osteoblasts located close to the endosteum. It regulates the HSC pool size by regulating HSCs quiescence. Further investigating a role of OPN in aging, a significant decrease in expression (by real time PCR) and secretion (by ELISA assay) of OPN in aged stroma was detected, with a significant decrease of the frequency of CD45-OPN+ stroma cells in aged mice. We next tested whether the decrease of OPN in stroma upon aging might be causatively linked to aging of HSCs. In vitro trans-well co-culture experiments of young and old hematopoietic stem and progenitor cells (HSPCs) on young, old and OPN KO stroma cells demonstrated a reduction of the number of LSK cells (Lin-Sca1+c-kit+) after 2 days in the presence of old and OPN KO endosteal stroma cells compared to cells co-cultured on young stroma. Heterochronic transplants of young and old BM cells into young, old and OPN KO mice revealed that an aged environment supports aging-associated lineage skewing of HSCs (myeloid over lymphoid), a decrease in overall engraftment and an increase in the frequency of long term HSCs (LT-HSCs). OPN KO recipients, similarly to aged recipients, also presented with a significant decrease in stem cell engraftment but an increase of the frequency of LT-HSCs, implying a causal role for reduced OPN in aged stroma for regulating aging-associated phenotypes of HSCs. We re-

cently reported that the majority of young HSCs is polar for planar cell polarity proteins and tubulin as well as for acetylated HistoneH4 at Lysine16 within the nucleus (AcH4K16), while the majority of aged HSCs is apolar. Finally we thus investigated the polarity status of LT-HSCs from each of the experimental group of the heterochronic transplants by single cell immunofluorescence staining. Interestingly young and thus polar LT-HSCs, when transplanted into either aged or OPN KO mice turned apolar for tubulin and AcH4K16, while young HSCs transplanted in young mice remained polar. Therefore, aging-associated reduction in OPN expression in stroma implies a novel and critical role for HSCs aging.

W-1157

THE ROLE OF ACTIVATED LEUKOCYTE CELL ADHESION MOLECULE (ALCAM / CD166) IN HEMATOPOIESIS AND HEMATOPOIETIC STEM CELL NICHE

Brahmananda Reddy Chitteti, Michihiro Kobayashi, Yinghua Cheng, Peirong Hu, Bradley Poteat, Hal E. Broxmeyer, Louis M. Pelus, Melissa A. Kacena, Edward F. Srour
Indiana University School of Medicine, Indianapolis, IN, United States

Hematopoietic stem cell (HSC) fate is controlled by ligand-counter ligand interactions that are expressed independently on HSC and hematopoietic niche (HN) elements. To date, not a single surface marker has been identified as a common marker expressed on murine and human HSC and on cells of the HN. We previously demonstrated that CD166 expression tracks the maturational status of osteoblasts (OB) and promotes higher hematopoietic enhancing activity (HEA). In co-culture studies, hematopoietic potential of HSC declined precipitously with increasing maturational status of OB concomitant with loss of CD166 expression. Fractionation of OB with lineage, Sca1, osteopontin (OPN), CD166, CD44, and CD90 revealed that OB with high Runx2, low osteocalcin expression, and with Lin-Sca1-OPN+CD166+CD44+CD90+ phenotype maintain higher in vitro and in vivo HEA compared to CD166- counterparts. Given that CD166 can mediate homophilic cell-cell interactions, we investigated the role of CD166 in identifying HSC and the impact of CD166 on hematopoiesis, HSC engraftment, and the HN. Interestingly, CD166+, but not CD166- fractions of murine and human repopulating HSC identified by a rigorous hierarchical classification for each species mediated robust long-term engraftment. In the murine system, Lineage- Sca1+ ckit+ (LSK) CD48-CD150+CD166+ cells mediated significantly higher chimerism 4 months post-transplantation (PT) compared to LSKCD48-CD150+CD166- cells suggesting that CD166 identifies long-term repopulating cells beyond what is possible with SLAM markers. Similarly, in the human system, cord blood-derived Lin-CD34+CD38-CD49f-CD166+ cells and Lin-CD34+CD38-CD49f+CD166+ cells engrafted 16 weeks PT in conditioned NSG mice significantly higher than Lin-CD34+CD38-CD49f+CD166- cells demonstrating that the CD166+ but not the CD166- fraction of CD34+Lin-CD38- cells contains long-term engrafting human HSC. In CD166 knockout (KO) mice, numbers of LSKCD48-CD150+ cells in the marrow and Lin-CD48- cells in circulation were significantly reduced relative to wild-type (WT) controls. LSK cells from CD166-/- mice failed to engraft in lethally irradiated WT recipients. Short-term repopulating cells engrafted efficiently in KO mice 1mo PT, but declined substantially thereafter demonstrating that the CD166-/- HN cannot support long-term repopulating cells. We assessed the impact of homophilic CD166 interactions on the HEA of OB using a co-culture system and demonstrated that high HEA was detected when both OB and LSK cells expressed CD166. Reconstitution kinetics data and survival of KO mice under hematopoietic stress conditions suggested that CD166-/- HSC have an intrinsic self-renewal capacity precluding them from both rapid proliferation and expansion and maintenance of the stem cell pool in the HN. Since Stat3 has 3 binding sites on the CD166 promoter, we examined the relationship between expression of Stat3 and CD166. Pharmacologic inhibition of Stat3 expression led to a simultaneous inhibition of CD166 expression. Overall, our data illustrate that CD166 is a universal marker of both murine and human HSC and OB within the HN and suggest that CD166 may modulate HSC-niche interactions and impact stem cell fate. The conserved homology between murine and human CD166 provides an excellent bridge between human and murine studies for efficient translational investigations and interventions for enhancing stem cell engraftment and clinical utility.

W-1158

DEVELOPMENT AND CHARACTERISATION OF A DYNAMIC EX VIVO BONE MARROW (BM) NICHE

Kathryn Futrega[1], Bill Lott[2], Kerry Atkinson[2], Michael R. Doran[1]
[1]Translational Research Institute (TRI), Brisbane, Australia, [2]Institute of Health and Biomedical Innovation (IHBI), Brisbane, Australia

Background: Ex vivo hematopoietic stem cell (HSC) niche recapitulation and HSC self-renewal remain among the holy grails of stem cells biology. Despite decades of laboratory and clinical research, the expansion of human HSC, capable of contributing to long-term engraftment in transplant recipients, has not been achieved. At present, the most promising HSC culturing techniques are cytokine/growth factor-supplemented liquid suspension cultures and 2-dimensional (2D) monolayer co-cultures. Co-cultures are intended to mimic the multicellular HSC niche environment, but their 2D geometry does not effectively mimic native cellular organization and ultimately this compromises the quality of the mimic. Our group has developed the first high-throughput, scaffold-free 3-dimensional (3D) HSC co-culture system. We use an in-house fabricated microwell surface to assemble hundreds-to-thousands of multicellular microaggregates, each containing 100 bone marrow (BM) derived mesenchymal stromal cells (MSC) and 10 human umbilical cord blood (UCB)-derived CD34+ cells - we term these mimics "micromarrows". Herein, we contrast the capacity of this 3D niche mimic to expand/maintain umbilical cord blood-derived CD34+CD38- cells relative to conventional 2D co-cultures.
Methods: The microwell surface was fabricated from polydimethylsiloxane (PDMS) and modified with an electrostatic multilayer to prevent cellular adhesion to the PDMS surface. The micromarrows were assembled with a ratio of 100 MSC to 10 CD34+ cells. Control 2D co-cultures were performed in parallel by seeding the same number of MSC and CD34+ cells in fibronectin-coated wells. Co-cultures were maintained for 7 days in a 2% O2, 5% CO2 atmosphere in serum-free X-Vivo 15 medium (Lonza), supplemented with a minimal amount of cytokine (10 ng/ml SCF). Furthermore, we tested the effect of growth factors that act indirectly, via MSC, on hematopoietic cells in 2D and 3D co-cultures and determined the number of CD34+CD38- cells. Cultures were characterized using fluorescence flow cytometry and confocal microscopy.
Results: Our results indicate that the scaffold-free 3D co-culture system supports the expansion of UBC-derived CD34+ cells and exhibits significantly more expansion of the CD34+CD38- cell population, compared to 2D co-cultures. During the first 3 hours of 3D co-culture, spontaneous cell aggregation occurs with hematopoietic cells organizing within the bulk MSC microsphere. Over the subsequent culturing period, the bulk MSC aggregate continues to condense, while hematopoietic cells 'spill' out of the microsphere as they migrate and/or proliferate, forming a proximal expanding cell pool. The addition of certain growth factors that act on MSC to co-cultures resulted in comparable CD34+ expansion in both 2D and 3D co-cultures; however, the CD34+CD38- population was depleted at a much lower MSC density in 2D compared to 3D.
Conclusion: Preliminary results suggest that 3D co-culture is a promising expansion platform for HSCs and may be a promising platform for BM niche recapitulation. The indirect effect of HSC differentiation factors is ameliorated in 3D co-cultures, suggesting this co-culture platform may be suitable for preserving more primitive HSC populations over long culturing periods. Moreover, this culturing system may serve as a tool to study complex HSC-MSC interactions ex vivo, such as those that drive HSC quiescence, proliferation, differentiation, migration and apoptosis.

W-1161

HETEROGENEITY OF YOUNG AND AGED MURINE HEMATOPOIETIC STEM CELLS REVEALED BY QUANTITATIVE CLONAL ANALYSIS USING CELLULAR BARCODING

Evgenia Verovskaya, Mathilde Broekhuis, Erik Zwart, Martha Ritsema, Ronald van Os, Gerald de Haan, Leonid Bystrykh
European Research Institute for the Biology of Ageing, University Medical Centre Groningen, Groningen, Netherlands

The number of hematopoietic stem cells (HSCs) that simultaneously contribute to blood formation, and dynamics of their clonal contribution is a matter of on-going discussion for the last five decades. Recent discoveries of heterogeneity of differentiation, migration and cycling behavior within the HSC pool further complicate this question. It is unclear how different HSC behaviors co-manifest within the same animal, what the number of active HSCs at a mo-

ment of time is and what the dynamics of polyclonal hematopoiesis upon aging is. Here we utilized combination of cellular barcoding technique and multiplex deep sequencing detection for high-resolution, quantitative, sensitive analysis of clonal fluctuations in hematopoietic system. Using this system, we studied clonal behavior of hundreds of young and old hematopoietic clones in mono-, oligo- and polyclonally repopulated mice and addressed different levels of HSC heterogeneity. Blood analysis showed that the majority of transplanted clones stably contributes to hematopoiesis over time, however clonal output in granulocytes, T cells and B cells is substantially different. While the clonal repertoire remained consistent from 12 up to 54 weeks post-transplant, contributions of the clones were dynamically changing, most of the clones either growing or declining in time. We further analyzed the population of primitive cells in the bone marrow and observed that HSC clones were mosaically distributed throughout the skeleton of the mice. Comparison of old and young HSCs revealed that the pool of old HSCs is composed of multiple small clones while young HSC pool can be dominated by a few clones. These data supports the theory of clonal stability of hematopoiesis, and at the same time demonstrate that this process is dynamic. We confirm that cellular barcoding analysis is instrumental in addressing multiple levels of heterogeneity in clonal HSC behavior in polyclonal system.

W-1162

THE FRIZZLED DOMAIN FROM COLLAGEN XVIII (FZC18) CONTROLS HEMATOPOIESIS AND MYELOIDOGENESIS IN MICE

Valerio Izzi, David Vicente, Inderjeet Kaur, Raman Devarajan, Heli Ruotsalainen, Mari Aikio, Hanne-Kaisa Honkanen, Ritva Heljasvaara, Taina Pihlajaniemi
Center for Cell-Matrix Research and Biocenter Oulu, University of Oulu, Oulu, Finland

Collagen XVIII (Col18), characterized by the antiangiogenic/tumor-suppressive endostatin domain at its C-terminus, occurs as three N-terminal variants. The corresponding gene has two promoters, promoter 1 encoding the shortest variant and promoter 2 the middle and longest variants. The longest variant is characterized by a frizzled-like domain (FZC18), known to be shed from full-length Col18, and shown to act as a soluble inhibitor of the Wnt3a/beta-catenin pathway. In view of the known importance of Wnt3a in hematopoietic stem cell (HSCs) development and myeloidogenesis, and the fact that Col18 is expressed in the bone marrow, we decided to investigate the effects of lack of Col18 on mouse hematopoiesis.
Using total and promoter-specific knockout mice for Col18, as well as mice overexpressing FZC18 under the keratin 14 (K14) promoter, the whole hierarchy of HSCs was screened for alterations in the numbers of self-renewable HSCs (srHSCs), multipotent precursors, myeloid and lymphoid precursors and mature bone marrow myeloid cells. The same mouse cohorts were also screened for differences in blood and spleen leukocyte counts and for embryonic HSCs at different developmental stages. Furthermore, in vitro long-term bone marrow cultures and myeloid cells cultures were adopted to clarify the role of FZC18 domain in hematopoiesis and myeloidogenesis.
Our data show for the first time a role for Col18 in hematopoiesis, as witnessed by a significant increase in HSCs in the knockout mice, both in the whole bone marrow as well as at specific stages during embryonic development. This effect was found to be more pronounced in promoter 2 knockout mice, recoverable in vitro with purified FZC18 protein, and totally absent in knockout mice overexpressing FZC18. As a consequence of HSCs impairment, a significant hematopoietic reprogramming towards myeloid progenitors was observed, which resulted in a wide increase of monocyte and granulocyte counts in the blood accompanied by a marked decrease in immature myeloid cells (IMCs) within the bone marrow.
Altogether, specific ablation of the middle and longest Col18 variants leads to an increase in srHSCs which reflects on peripheral monocyte and granulocyte accumulation, without any appreciable effect on the specific compartment of the immune response. These data thus provide evidence of novel roles for Col18 and its FZC18 variant, capable of binding Wnt molecules, as endogenous controllers of myeloidogenic commitment during HSC development.

W-1163

AUTOLOGOUS UMBILICAL CORD BLOOD FOR REGENERATIVE MEDICINE APPLICATIONS

Katherine S. Brown[1], Heather L. Brown[1], David T. Harris[2]

[1]Scientific and Medical Affairs, Cbr Systems, Inc., San Bruno, CA, United States, [2]Department of Immunobiology, University of Arizona, Tucson, AZ, United States

Background:
For over 20 years, umbilical cord blood (UCB) has been a source of hematopoietic stem cells (HSCs) for use in transplant medicine to treat malignancies, blood disorders and genetic diseases. Newborn stem cells are now being researched in regenerative medicine applications to repair damaged or diseased tissues outside of the blood and immune system. Preclinical studies have shown that UCB contains a heterogeneous population of stem cells. Furthermore, cord blood stem cells have been shown to migrate to injured tissues, secrete therapeutic factors and modulate the immune system. These findings suggests that UCB stem cells may have potential for a variety of regenerative medicine applications. Autologous cell sources, such as family banked UCB, are preferred in regenerative medicine, as they do not have immune complications nor require conditioning regiments.
Objective:
To assess the indications and characteristics of UCB units released by a family stem cell bank for autologous use in regenerative medicine.
Methods:
UCB was collected from consenting mothers who elected to family bank. The UCB was transported to Cord Blood Registry (CBR)'s processing facility in Tucson, AZ. The whole blood product was processed to remove the plasma and majority of the red blood cells and other non-engrafting cells. The processed UCB was stored in the vapor-phase of liquid nitrogen in dewars at -195C until requested for use. Prior to release of the unit, viability and percent CD34+ (or CFU) were assessed from an attached segment by the treating institution. Viability assays varied by institution. Infusions were performed under research protocols at major medical institutions.
Results:
Through January 30th of 2013, CBR released 236 UCB units. 72 (30.5%) units were released for use in a transplant while 164 (69.5%) units were released for autologous regenerative medicine therapies including: cerebral palsy (90), traumatic brain injury (4), autism (4), type 1 diabetes (11) and other conditions. 31 units used for regenerative medicine were released for patients participating in clinical trials. The mean storage time for autologous units was 36.12 months, the mean viability was 95.1% and the mean CD34+ cell count was 0.52%.
Conclusion:
UCB is increasingly being utilized as a source of stem cells in cellular therapies for treatment of a wide range of disorders. Clinical trials are evaluating the safety and efficacy of UCB in regenerative medicine applications such as cerebral palsy, traumatic brain injury, autism and pediatric stroke. The data here support that emerging therapies utilizing autologous cord blood stem cells are a significant and growing percentage of all the treatments facilitated by a family bank. Family banks provide a valuable resource for storing UCB for future autologous or related allogeneic stem-cell based therapies and are facilitating a new era of clinical research focused on regenerative medicine.

W-1164

A SUB-POPULATION OF EXPANDED HEMATOPOIETIC PROGENITOR CELLS THAT RETAIN HIGH ALDEHYDE DEHYDROGENASE ACTIVITY DEMONSTRATE ISLET REGENERATIVE PROPERTIES

Ayesh K. Seneviratne[1], Gillian I. Bell[2], David M. Putman[1], David A. Hess[1]

[1]Physiology and Pharmacology, University of Western Ontario, London, ON, Canada, [2]Robarts Research Institute, London, ON, Canada

357 million individuals worldwide currently have diabetes and >90% of these patients will develop severe vascular complications shortening their life expectancy. We have previously identified that human umbilical cord blood (UCB) cells with high aldehyde dehydrogenase activity ($ALDH^{hi}$), a conserved stem cell function of multiple progenitor lineages, can stimulate β-cell proliferation and islet vascularization after intrapancreatic transplantation into mice with steptozotocin (STZ)-induced diabetes. However, UCB $ALDH^{hi}$ cell are rare (<0.5% of MNC), and expan-

sion of these cells under clinically applicable regimens will be required to treat patients with diabetes. Interestingly, $ALDH^{hi}$ stem cells are primarily composed of hematopoietic progenitor cells (HPC, 93.3 ± 1.9% $CD45^{+}$, N=3) that are efficiently expanded in short-term culture. Thus, the purpose of these studies was to characterize the islet regenerative capacity of UCB-derived $ALDH^{hi}$ cells after clinically applicable serum-free expansion under hematopoietic conditions *ex vivo.* We hypothesized that culture-expanded HPC that retain high-ALDH activity would stimulate islet regeneration via paracrine mechanisms after transplantation *in vivo*. $ALDH^{hi}$ cells were first isolated from human UCB (n=8) using fluorescence activated cell sorting (FACS), and expanded in serum-free media supplemented with hematopoietic cytokines (10ng/mL rhSCF, rhFLT3L, rhTPO). Using these conditions total cell number was increased by 18.6±2.6 fold within 6 days, and 1 in 8 expanded progeny possessed multipotent hematopoietic colony forming capacity in methylcellulose media (n=4). Interestingly, a distinct sub-population (13.2±2.7%) of expanded progeny retained high ALDH-activity characteristic of hematopoietic progenitor cells ($ALDH^{hi}$-HPC). $ALDH^{hi}$-HPC also expressed primitive myeloid (99.9±0.1% $CD33^{+}$) and hematopoietic progenitor cell markers (77.6±4.2% $CD34^{+}$, 48.2±7.7% $CD133^{+}$) without the acquisition of mature monocyte (0.4±0.1% $CD14^{+}$) or macrophage markers (0.6±0.2% $CD11b^{+}$). Next, 6-day expanded HPC bulk progeny were intravenously (iv)-transplanted into STZ-treated NOD/SCID mice, and blood glucose levels were monitored to evaluate hyperglycemia reduction *in vivo*. Surprisingly, mice transplanted with expanded bulk HPC (n=9) remained severely hyperglycemic, similar to PBS-injected controls (n=6). Therefore, expanded HPC progeny were re-selected for low versus high ALDH activity ($ALDH^{lo}$-HPC vs $ALDH^{hi}$-HPC), prior to iv-transplantation. Remarkably, mice transplanted with $ALDH^{hi}$-HPC (n=5) demonstrated reduced systemic glucose levels and improved glucose tolerance, compared to mice transplanted with $ALDH^{lo}$-HPC (n=6). Although immunohistochemical characterization of the islet regenerative programs stimulated by these cells is ongoing, these preliminary finding suggest that expanded HPC that retain high ALDH activity possess robust islet regenerative capacity *in vivo*. This data identify expanded $ALDH^{hi}$-HPC as a novel cell population for clinical application, and provide strong justification for the further development of expansion, re-selection, and transplantation technologies using UCB-derived HPC for islet regenerative therapies in patients with diabetes.

W-1165

NFI GENES ARE NOVEL REGULATORS OF HEMATOPOIETIC- AND PROGENITOR CELL REPOPULATING POTENTIAL

Per Holmfeldt, Jennifer Pardieck, Shannon McKinney-Freeman
St. Jude Children's Research Hospital, Memphis, TN, United States

Hematopoietic stem cells (HSCs) are both necessary and sufficient to sustain the complete blood system of vertebrates. HSCs are heavily utilized in the clinic for bone marrow transplants (BMTs) to treat a variety of diseases. However, a lack of suitable donors and inefficiency in recipient engraftment currently limit this life saving therapy. To improve BMT regimens, a better understanding of regulators of HSC BM engraftment is required.
The transcription factor Nfix, a member of the nuclear factor I (Nfi) family of transcription factors, is highly expressed in HSCs. This fact suggests that Nfix may play a novel role in regulating HSC function. To test this hypothesis, HSCs were enriched from adult BM (Lineage-, c-kit+, Sca-1+ (LSK) cells) and then transduced with lentiviruses carrying shRNAs targeting Nfix. Twenty-four hours post-transduction, cells were injected into lethally irradiated mice along with untransduced BM LSK competitor cells congenic at the CD45 allele. The peripheral blood of recipient mice was then analyzed periodically over 16 weeks for engraftment of the Nfix-depleted cells. Depletion of Nfix by two independent shRNA (confirmed by Western blot analysis to deplete Nfix protein levels to <20% of baseline) resulted in a significant decrease in the repopulating activity of BM LSK cells relative to LSK cells transduced with either of two independent control shRNAs. As early as two weeks post-transplant, a 22% +/- 5% ($p=0.03$) reduction in repopulating activity was observed. By 16 weeks post-transplant, this reduction in repopulating potential had gradually increased to 55% +/-8% ($p<0.0001$) in four independent experiments. Lineage analysis of peripheral blood of recipients showed no significant differences in the distribution of the major blood lineages derived from LSK cells transduced with Nfix- specific shRNAs compared to controls. Depletion of either Nfia or Nfic, two Nfi family members also expressed by HSCs, in LSK cells via shRNAs led to a similar loss in LSK repopulating potential, sug-

gesting a general role for Nfi genes in the regulation of hematopoietic stem and progenitor repopulating potential. When the BM of recipients transplanted with Nfix- depleted LSK cells was examined 4 and 16 weeks post-transplant, a loss of phenotypic HSCs (LSK/CD150+/CD48-) relative to controls was evident. Thus, the observed decrease in repopulating activity likely occurs at the level of HSCs and multipotent progenitors
Analysis of early engraftment revealed that Nfix-depleted LSK cells established in the BM but failed to expand due to excessive apoptotic cell death. Critically, in vitro analysis of BM LSK expansion in liquid culture revealed no differences in the activity of LSK cells transduced with Nfix-specific shRNAs compared to controls. Thus, it is unlikely that the observed decrease in BM repopulating activity is due to general cytotoxicity resulting from Nfix-depletion. Moreover, gene expression profiling revealed that a number of previously described HSC regulators, Evi1, Mpl and Prdm16, were downregulated in Nfix-depleted LSK cells. Current studies aim to elucidate if any of these genes has a direct causative role for the observed phenotype following Nfix depletion and whether they are direct transcriptional targets of Nfix.
In summary, we have for the first time established a role for the Nfi gene family in HSC biology, as evident by a decrease in BM repopulating activity in HSCs depleted for the expression of these genes.

W-1166

TARGETED INHIBITION OF INNATE IMMUNOSURVEILLANCE TO PROMOTE THE ENGRAFTMENT OF HEMATOPOIETIC STEM CELLS

Nathaniel B. Fernhoff, Irving L. Weissman
Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA, United States

Despite the enormous therapeutic potential of bone marrow transplants, the procedure carries significant risks of morbidity and mortality. Some of the risks are associated with the cytoreductive conditioning regimen that clinicians must use to clear niche space and promote donor cell engraftment. Without high-dose radiation or chemotherapy, donor bone marrow cells fail to engraft in host bone marrow. Other risks are associated with transplanting donor T cells, which cause an immune response against host tissue. However, depleting T cells from transplants causes a further increase in engraftment failure. Therefore, there is a pressing medical need to develop new methods to increase donor cell engraftment in bone marrow transplants. We are developing a novel strategy of therapeutic delivery that may find wide application in transplant biology. CD47 is a ubiquitously expressed “don’t eat me signal” that negative regulates macrophage activation and phagocytosis. Cell surface CD47 expression is a necessary factor for hematopoietic stem cell engraftment in wild-type mice and a sufficient factor to promote the engraftment of MOLM-13 cell line in immunodeficient mice. Because of the risks associated with gene therapy, we are capitalizing on protein-based strategies to artificially increase CD47 protein levels on the donor cell surface. Using biotin / streptavidin crosslinking, we have successfully increased cell surface display of CD47 and we are actively characterizing the effect on bone marrow transplants and hematopoietic stem cell transplants with various conditioning regimens. We propose to treat donor bone marrow cells ex vivo with both of these strategies in order to evade innate immunosurveillance and promote donor cell engraftment.

W-1167

ENDOTHELIAL BLOOD BONE MARROW BARRIER REGULATES NORMAL AND LEUKEMIC STEM AND PROGENITOR CELL TRAFFICKING AND MAINTENANCE

Tomer Itkin[1], Shiri Gur-Cohen[1], Aya Ludin[1], Karin Golan[1], Alexander Kalinkovich[1], Orit Kollet[1], Ralf H. Adams[2], Tsvee Lapidot[1]
[1]Immunology, Weizmann Institute of Science, Rechovot, Israel, [2]Department of Tissue Morphogenesis, Max Planck Institute for Molecular Biomedicine, Münster, Germany

Bone marrow (BM) endothelial cells (BMEC) serve as ‘niche’ cells

for both hematopoietic and mesenchymal stem and progenitor cells (HSPC/MSPC). Yet, BMEC-mediated synchronization of HSPC bi-directional trafficking through the blood-bone marrow-barrier (BBMB) and of stem cell maintenance is poorly understood.
In vivo treatment with the angiogenic cytokine FGF-2 resulted in increased BM HSPC/MSPC numbers and repopulation potential, while reducing peripheral blood (PB) HSPC numbers and their repopulation potential from the PB. FGF-2 treated mice had reduced BBMB permeability evaluated by dye penetration and incorporation, and reduced HSPC BM homing. We have generated endothelial specific inducible FGF receptor knock-out (eFGFR1/2 KO), using VE-Cadherin-CRE^{ERT2} and FGFR1$^{flx/flx}$/FGFR2$^{flx/flx}$ transgenic mice). Importantly, eFGFR1/2 KO mice exhibited reduced BM stem cell levels. FGF-2 treatment was able to increase stem cell levels only in WT BM, but not in the BM of eFGFR1/2 KO mice. FGF-2 treated eFGFR1/2 KO mice failed to exhibit increased HSPC retention and reduced BM homing like in WT treated mice. These results suggest that endothelial specific activation of FGF signaling regulates the BBMB control of HSPC bi-directional trafficking. Thus, hampering endothelial FGF signaling interferes with stem cell maintenance and expansion, while increasing BBMB permeability. In addition we have observed that Nestin-GFP MSPC co-localize with non-permeable blood vessels. Mechanistically, FGF-2 treatment resulted in decreased BM MMP-9 activity combined with upregulated Timp-1 (MMP-9 inhibitor) mRNA levels. Furthermore, FGF-2 treatment activated AKT, and reduced NO and ROS content in BMEC. Importantly, permeability regulating endothelial junction molecules such as VE-Cadherin and ZO-1 were upregulated following FGF-2 treatment. Administration of neutralizing VE-Cadherin antibodies efficiently increased HSPC egress by increasing BBMB permeability. These results suggest that interfering with endothelial adhesion interactions alone increases HSPC trafficking. Examination of eNOS KO and Timp-1 KO mice revealed that HSPC bi-directional trafficking was decreased in eNOS KO mice and increased in Timp-1 KO mice. In the BM, primitive, repopulating HSPC numbers were increased in eNOS KO mice and decreased in Timp-1 KO mice. Of interest, we observed that chimeric immune-deficient mice transplanted with malignant human pre-B ALL cells, exhibited reduction in BBMB permeability. We suggest that leukemic cells stimulate BMEC in order to support their own maintenance, expansion and chemoresistance via reducing BBMB permeability, and CXCL12 while increasing SCF levels. This suggests that human leukemias can modify the BBMB to a malignant-supportive microenvironment and that BBMB targeting could hamper leukemia expansion and protection, to improve clinical chemotherapeutic protocols.
In conclusion, our findings reveal that normal and malignant stem cell migration and development are dynamically regulated by BMEC. We suggest that BMEC balance their dual roles as stem cell niches, and as an anatomical barrier synchronizing HSPC bi-directional trafficking. Thus, unbalanced BBMB function towards either stem cell trafficking or maintenance will come at the expense of the other.

W-1168

DEVELOPMENT OF AN OPTIMAL CULTURE SYSTEM FOR INDUCTION OF FUNCTIONAL HUMAN NEUTROPHILS FROM SERUM-FREE EXPANDED HEMATOPOIETIC STEM CELLS

Chao-Ling Yao, Yu-Shuo Hu
Department of Chemical Engineering and Materials Science, Yuan Ze University, Chung-Li City, Taiwan

Hematopoietic stem cells (HSCs) from bone marrow, peripheral blood and cord blood are widely used in transplantation after cancer chemotherapy. Among them, there are several limitations of cord blood hematopoietic stem cell transplantation, such as slow recoveries of neutrophils and platelets. The aim of this study is to develop an optimal neutrophil induction culture system to increase the number of neutrophils in vitro, to accelerate the recovery of patient's autoimmune system after cord blood HSC transplantation and to overcome the limitation of clinical application.
In this study, HSCs were isolated from cord blood and were expand by a serum-free HSC expansion system (SF-HSC medium) that was developed previously by our lab. Then we screened five of cytokines (SCF, G-CSF, GM-SCF, IL-3 and IL-1) and optimized the concentration of cytokines that can effectively induce serum-free expanded HSCs into CD66+/MPO+ cells. In addition, we also checked CD66+/MPO+ cells by neutrophil-associated surface antigens, such as CD11b, CD13, CD15, CD16, CD33, CD64, TLR2 and TLR4, and neutrophil-specific functional assays, such as

phagocytosis assay, chemotaxis assay and cellular ROS production assay. Finally, we confirmed induced cells were functional neutrophils.
In this study, we have successfully established a neutrophil induction medium that can effectively induce HSCs into mature and functional neutrophils in vitro. We believe that combination of SF-HSC medium and neutrophil induction medium can generate a large amount of functional neutrophils and provide a promising cell source for basic research and clinical application in the future.

W-1171

DYNAMICS OF LONG-TERM HSPC SUBTYPES IN NON-HUMAN PRIMATES REVEALED BY A DECADE-LONG CLONAL TRACKING EXPERIMENT

Sanggu Kim[1], Namshin Kim[2], Angela P. Presson[1], Mark E. Metzger[3], Gay M. Crooks[1], Dong-Sung An[1], Robert E. Donahue[3]

[1]University of California, Los Angeles, Los Angeles, CA, United States, [2]Korean Bioinformatics Center, Daejeon, Korea, Republic of, [3]National Heart, Lung and Blood Institute, Bethesda, MD, United States

Hematopoietic stem cell and progenitor cell (HSPC)-based genetic therapy to treat HIV or other previously incurable diseases is becoming increasingly realistic. The composition of the primate HSPC pool nor the regenerative potential of cells composing the HSPC pool is, however, yet to be clearly understood due partly to the lack of appropriate in vivo models and experimental tools. Here, we report detailed behavior patterns of repopulating HSPCs in four rhesus macaques transplanted with autologous CD34+ cells 4 to 12 years ago after myeloablative conditioning. Prior to transplantation, cells were transduced with either control lentivirus vectors expressing EGFP or vectors expressing both EGFP and shRNA against the HIV-1 co-receptor, C-C chemokine receptor type 5 (CCR5). All animals showed normal hematopoietic recovery and maintained stable marking in all tested blood lineages. In order to study the behavior of engrafted HSPCs, vector marked clones in serially collected blood as well as different blood lineages were analyzed using a high-throughput quantification assay. The assay, involving large-scale vector integration site (VIS) sequencing and bioinformatics analysis of VIS sequences, allowed sensitive quantification of individual clones in a polyclonal blood cell pool. Unlike traditionally used techniques that employed bulk populations, our clonal marking tracked the functional behavior of a single cell. Analysis revealed thousands of HSPC clones expanded sequentially over time in each animal, clustered into groups with different kinetics of repopulation, maintaining the total marked blood cells at a relatively stable level over the years. While short-term repopulating clones provided rapid and transient immune recovery, long-term engrafted clones gradually expanded and became the main source of repopulating cells starting from 8 - 13 months post-transplantation. Importantly, consistent with recent discoveries in murine HSC studies, these long-term clones were also distinctively grouped into “myeloid-biased”, “lymphoid-biased”, and “balanced” subtypes based on their unique lineage output potentials. Clones with more balanced lineage potentials, accounted for only 4 − 10% of the identified clones, yet predominated, contributing up to 25 − 71 % of total repopulating cells in each animal. No notable effect of CCR5 shRNA expression upon clonal repopulation was observed. Our long-term clonal tracking revealed new insights regarding the biology of primate HSPCs, providing a potential frame of reference for future HSPC based gene therapy clinical trials.

W-1172

HIERARCHICAL CHANGES OF HUMAN HEMATOPOIETIC STEM CELL DIFFERENTIATION DURING ONTOGENY

Sasan Zandi, Faiyaz Notta, Stephanie Dobson, Naoya Takayama, John E. Dick
UHN, Toronto, ON, Canada

Hematopoietic stem cells (HSCs) undergo phenotypic, functional, and anatomic changes over the course of ontogeny. Murine studies show differences in surface markers, cytokine dependency and self-renewal/differentiation capacity of stem and progenitors between the fetus and adult. The system tends to produce more lymphoid and megakaryocytic-erythroid (Mk/E) cells in fetal stage but changes in favor of more myeloid biased outputs in adults. During aging, this myeloid (My) bias correlates with increased incidence of myeloid malignancies such as acute myeloid leukemia (AML). Our understanding of the dynamics of human hematopoiesis during ontogeny is very limited due to the absence, until recently, of consistent methods to clonally resolve human HSC and progenitors. Using these advanced sorting methods and a highly efficient single cell functional assay that supports My, E and Mk differentiation, we traced the lineage potential of nearly 4000 deposited single cells from highly purified HSC and progressively restricted progenitor populations from across human development. There was a progressive reduction in the proportion of HSC and multipotent progenitor cells between fetal liver to adulthood. Strikingly in the adult, all multipotent cells are relegated to the CD34+CD38- stem cell compartment. Although CMP are commonly considered as CD34+CD38+, our single cell analysis of adult BM showed that more than 95% the cells in CD34+CD38+ compartment are unipotent, revealing important developmental changes. We then used CD71 (transferrin) and cMPL (TpoR) to identify new classes of hematopoietic progenitors in human FL, cord blood (CB) and adult BM. While >90% of CD34+CD38+CD71+cMPL+ cells in adult BM are committed erythroid progenitors, in FL and CB more than 40% of them are multipotent. Noticeably Mk cells can be generated from several different multipotent progenitors indicating multiple branching points for Mk in FL and CB. In-vivo repopulation assays in NSG mice confirmed that CD71 and cMpl expression marks erythroid-committed progenitors with varying degrees of proliferation capacity that can be resolved based on variation in CD45RA and Flt3 expression. To gain further insight into molecular mechanisms governing early commitment of erythroid and megakaryocyte lineage, we used Fluidigm to quantify the expression of lineage-associated genes in single progenitors that expressed a combination of these surface markers. We detected co-expression of EpoR, TpoR and GATA1 in more than 90% of CD71+cMpl+ progenitors from FL, while >85% of adult BM CMPs expressed Mpo and GCSFR but lacked erythroid associated genes. Our gene expression data also showed that stemness programs extend much farther down into the hierarchy within the fetal stage where progenitors are more flexible to adopt E, Mk or My fates. It is only in the adult BM stage that progenitors make binary and distinct decisions.

W-1173

HUMAN HEMATOPIETIC STEM AND PROGENITOR CELLS INJECTED IN HUMANIZED NSG AND MITRG MICE ENGRAFT THE MENINGEAL COMPARTMENT

Mariangela Amenduni[1], Anthony Rongvaux[2], Richard A. Flavell[2], Flora M. Vaccarino[1]
[1]Child Study Center, Yale University, New Haven, CT, United States, [2]Department of Immunobiology, Yale School of Medicine, New Haven, CT, United States

Microglia, the resident macrophages of the CNS, are thought to participate in the pathogenesis of neurodevelopmental and neurodegenerative diseases. However the origin of brain microglia remains unclear. It has been recently shown that in mice microglia derive from primitive myeloid progenitors of the yolk sac and that these cells are able to penetrate the developing brain around embryonic day 8. On the contrary perinatal circulating hematopoietic precursors do not seem to significantly contribute to the pool of adult microglia.
This result is in contrast with previous studies showing that bone -marrow derived cells able to engraft the brain during neurodegeneration and inflammation and differentiate into microglia-like cells when bone marrow transplantation is preceded by irradiation-mediated immune ablation. To date there is no evidence that this is true also in humans. We tested whether human hematopoietic stem and progenitor cells (HSPCs; CD34+ cells isolated from human fetal liver) are able to differentiate into brain microglia. Our data indicate that HSPC transplanted in the liver of neonatal immunodeficient mice expressing multiple human cytokines required for myelopoiesis (M-CSF, GM-CSF, IL-3 and TPO) are not able to engraft the adult mouse brain; indeed, human cells positive for CD11b/Iba1 are mainly localized within the meninges. To address whether the blood brain barrier that is already formed after birth might have prevented human hematopoietic cells from entering the brain, we injected the HSPCs in embryonic mouse brain at E12; however, engrafted human cells positive for CD11b/Iba1 were localized in the meninges. Future research will reveal whether HSC are already committed toward a more differentiated state and/or lack brain specific- triggers that allow these cells to colonize the brain. Furthermore it would be interesting to understand whether

these cells are able to enter the brain after damage and whether they will display the same characteristics of the endogenous yolk sac-born microglia.
Knowing the unique origin of microglial cells will in fact contribute to better understand their role in neurological and psychiatric disorders and develop therapeutic strategies, since specific myeloid cells-from the yolk sac, bone marrow or blood could each be involved in shaping circuit development and pruning. These cells could also used to deliver specific compounds into the damaged brain.

W-1174

INCREASED MIR-223 EXPRESSION DYSREGULATES MYELOID DIFFERENTIATION IN PTPN11-ASSOCIATED JMML

Sonia Mulero-Navarro[1], Ana Sevilla[1], Angel Roman[2], Dung-Fang Lee[1], Sunita D'Souza[1], Jie Su[1], Christoph Schaniel[1], Brian Brown[1], Hélène Cavé[3], Marco Tartaglia[4], Ihor Lemischka[1], Bruce Gelb[1]
[1]Icahn School of Medicine at Mount Sinai, New York, NY, United States, [2]Instituto Cajal-Consejo Superior de Investigaciones Científicas, Madrid, Spain, [3]Department of Genetics, AP-HP-Robert Debré Hospital, and Paris-Diderot University, Paris, France, [4]Dipartimento di Ematologia, Oncologia e Medicina Molecolare, Istituto Superiore di Sanità, Rome, Italy

Somatic
mutations in *PTPN11*, encoding the
RAS/MAPK regulator SHP-2, cause juvenile myelomonocytic leukemia (JMML). Germ-line *PTPN11* defects cause Noonan syndrome
(NS), while specific inherited mutations cause NS/JMML. Hematopoietic cells differentiated from human induced pluripotent stem
cells (hiPSCs) harboring NS/JMML-causing *PTPN11*
mutations recapitulated JMML features including granulocyte macrophage
colony-stimulating factor hypersensitivity and increased fetal hemoglobin,
documenting sufficiency of the *PTPN11*
mutation for leukemogenesis. hiPSC-derived NS/JMML myeloid cells exhibited increased signaling
through STAT5 and up-regulation of miR-223 and miR-15a.
Reducing miR-223's function in NS/JMML hiPSCs normalized myelogenesis.
Micro-RNA target gene expression levels were reduced in hiPSC-derived myeloid
cells as well as in JMML bone marrow mononuclear cells, of which ~70%
of the patient samples also showed miR-223 and/or miR-15a up-regulation. The findings confirm the utility of studying
inherited human cancer syndromes with hiPSCs to understand early oncogenesis
prior to the accumulation of secondary genomic alterations and provide opportunities
for therapeutic target discovery and small molecule testing.

W-1175

THE ARYL HYDROCARBON RECEPTOR (AHR) REGULATES THE PRODUCTION OF BIPOTENTIAL HEMATOPOIETIC PROGENITOR CELLS

Brenden W. Smith[1], Sarah S. Rozelle[1], Amy Leung[1], Shirley K. Nah[1], Deborah French[2], Paul Gadue[2], Darrell N. Kotton[1], Gustavo Mostoslavsky[1], David H. Sherr[3], **George J. Murphy**[1]
[1]Boston University Center for Regenerative Medicine (CReM), Boston Univ School of Medicine, Boston, MA, United States, [2]Center for Cellular and Molecular Therapeutics, Children's Hospital of Philadelphia, Philadelphia, PA, United States, [3]Dept. of Environmental Health, Boston Univ School of Public Health, Boston, MA, United States

The evolutionarily conserved aryl hydrocarbon receptor (AhR), a member of the basic helix-loop-helix Per-ARNT-Sim family, has been studied for its role in environmental chemical-induced toxicity. Recent studies now demonstrate that the AhR may regulate the hematopoietic and immune systems during development in a cell-specific manner. To better understand the possible role of the AhR in hematopoiesis, we developed a novel, human, induced pluripotent stem cell (iPSC) platform based on the step-wise directed differentiation of hematopoietic progenitor cells. Our in silico analysis of transcriptional profiles of 71 primary human hematopoietic cell isolates indicated AhR-upregulation at both the hematopoietic stem cell and bi-potential megakaryocyte-erythroid progenitor (MEP) stages. This result, together with the absence of an in vitro model system enabling production of large numbers of primary human MEPs capable of differentiating into megakaryocytes (Mks) and erythroid lineage cells, motivated us to determine if AhR modulation could facilitate both MEP expansion and Mk and erythroid cell differentiation.

Our results indicate that AhR has a physiological and functional role in normal hematopoietic development, and that modulation of the receptor in bi-potential hematopoietic progenitors can direct cell fate. We demonstrate a novel methodology for the directed differentiation of pluripotent stem cells in chemically defined, serum and feeder-free culture conditions into MEPs capable of final specification into Mks and/or erythroid-lineage cells. The use of a non-toxic aryl hydrocarbon receptor agonist in our directed differentiation scheme significantly increases the number of MEPs and resultant cells. Following the addition of potent AhR ligands to our cultures, we observed exponential expansion of MEPs from fifteen thousand to one billion cells in two weeks, with the role of AhR in the MEP population confirmed using a specific AhR inhibitor. This logarithmic expansion of cells appears to be a function of decreased cell death and is consistent with previous studies which suggest that the AhR can control apoptosis. In addition to allowing for the exponential expansion of MEPs, our results demonstrate that modulation of AhR can direct cell fate, with AhR agonism permissive to erythroid differentiation and antagonism favoring Mk specification. Although erythropoietin (EPO) and thrombopoietin (TPO) stimulate RBC and Mk production respectively, AhR may play a cytokine-independent role in the specification of these lineages and warrants further study.

These results demonstrate a new platform for studying human red blood cell and MK development that allows for exponentially greater production of RBCs and Mks in comparison to existing methodologies. This strategy relies on the first of its kind definition of the role of the AhR receptor in normal hematopoietic development using specialized ligands in hematopoietic progenitor cells. A useful outcome for this work will be the utilization of this in vitro platform for the clinically relevant production of blood products. An iPSC-based system, such as the one described here in which sufficient numbers of cells can be produced, should facilitate future clinical adaptation involving the transfusion of iPSC-derived red blood cells and platelets without problems related to immunogenicity, contamination, or supply.

W-1176

IN VIVO TIME LAPSE IMAGING OF MOUSE BONE MARROW REVEALS DIFFERENTIAL NICHE ENGAGEMENT BY QUIESCENT AND PHYSIOLOGICALLY ACTIVATED HAEMATOPOIETIC STEM CELLS

Mark Scott[1], Narges Rashidi[1], Jens Kalchscmidt[1], Nico Scherf[2], Ingo Roeder[2], Kleoniki Gounaris[1], Murray Selkirk[1], **Cristina Lo Celso**[1]

[1]Life Sciences, Imperial College London, London, United Kingdom, [2]Institute for Medical Informatics and Biometry, Technical University Dresden, Dresden, Germany

Understanding the mechanisms linking stem cell-niche interaction and stem cell fate is critical for developing regenerative medicine approaches. The nature of such interactions between hematopoietic stem cells (HSC) and the bone marrow (BM) microenvironment has long been elusive due to the difficulty of penetrating bones for direct observation and the fluid nature of the hematopoietic tissue itself. Several functional studies based on ablating or overexpressing specific genes in the hematopoietic or distinct BM stroma compartments have highlighted the presence of an intricate and dynamic network of regulatory signals responsible for the crosstalk between HSC and the BM microenvironment. The question, however, remains open as to whether multiple, molecularly and functionally

distinct HSC niches exist within the bone marrow and whether HSC trafficking between them may be necessary to switch fate between quiescence and proliferation, self-renewal and differentiation.
To address this question, we developed an imaging technique combining two photon and confocal microscopy that allows in-vivo imaging of live transplanted hematopoietic stem and progenitor cells (HSPC) in mouse BM with single cell resolution. Using this technique we showed that engrafting long-term repopulating HSC (LT-HSC) localize near osteoblastic cells, while their progeny are more distal. Our results also highlight that localization of LT-HSC and their progeny near osteoblasts correlates with improved engraftment outcomes. Studies based on single time-point observations demonstrated that asynchronous HSPC proliferation initiates BM reconstitution, however did not provide information about long-term interactions between HSC and their BM niche (or niches), which are responsible for maintenance of balanced hematopoiesis. We therefore developed a new in vivo imaging experimental protocol, allowing time-lapse imaging of HSPC, leading us to uncover their differential abilities to engage with the BM microenvironment over time. Moreover, using a physiological model of HSC activation, we observed that changes in the nature of the interactions between stem cells and the BM microenvironment accompany switches in fate choice.

W-1177

A COMPARISION OF INTRAUTERINE HAEMOPOIETIC STEM CELL & DIRECT IN VIVO LENTIVIRAL THERAPIES FOR BETA THALASSEMIA MAJOR IN A MURINE MODEL OF FETAL THERAPY.

Niraja Dighe[1], Lay Geok Tan[1], Daren Sandikin[1], Simon Waddington[2], Michael Antoniou[3], Mahesh Choolani[1], Jerry Chan[1], Citra Mattar[1]

[1]Experimental Fetal Medicine Group, Department of Obstetrics and Gynecology,, National University of Singapore, Singapore, Singapore, [2]Gene Transfer Technology Group,, Institute for Women's Health, University College London,, London, United Kingdom, [3]Department of Medical and Molecular Genetics,, King's College London School of Medicine, Guys Hospital,, London, United Kingdom

AimsSevere haemoglobinopathies like the major thalassaemias cause perinatal complications including death, or chronic illness requiring life-long red cell transfusion. Prenatal stem cell transplantation has not succeeded in correcting the outcomes of these diseases thus far. Yet fetal therapy could possibly arrest pathogenesis early enough to allow perinatal survival, or to improve the severity of the disease such that transfusion independence is acquired. To realize the putative benefits of the fetal microenvironment that may facilitate genetic correction, we investigated the dual intrauterine strategies of haemopoietic stem cell transplantation (IUHSCT) and direct in vivo gene therapy (IUGT) designed to treat a murine fetal model of beta thalassaemia major. Our specific aims were to compare donor cell engraftment, vector biodistribution and phenotype improvement in treated progeny.
MethodsHbb/Th3+ males were backcrossed with C57BL/6 females on a CD45.2 background. IUHSCT fetuses were given $2x10^5$-$1x10^6$ cells of either murine fetal liver-derived CD45.1 mononuclear cells, or human fetal liver-derived HSCs intraperitoneally. Cells were treated with Diprotein A for CD26 inhibition just prior to transplantation. In the IUGT arm, F1 fetuses were given $5x10^6$ TU IV (via the vitelline vein) of a lentiviral vector (LV) LCR-β-globin. Injections were performed at E15-18. Weaned pups were analysed by genotype, blood smears and haematological indices. Chimerism was determined in peripheral blood in IUHSCT pups, and was studied in the liver, bone marrow and spleen at six months. IUGT pups were monitored by vector biodistribution and intracellular expression of human beta globin. Phenotype changes were studied in treated Hbbth3/+ offspring, while uninjected progeny served as controls.
Results In the IUHSCT arm, peripheral blood chimerism was observed at a median level of 0.54% (n=8, range 0.1-1.7%) with murine CD45.1 cells, while with human CD45 cells median engraftment was 0.33% (n=20, range 0.1-3.4%) at 8 weeks. Median levels of engraftment in bone marrow, liver and spleen (n=17) were 0.17% (range 0.1-0.54%), 1.05% (range 0.1-5.96%) and 0.16% (range 0.1-0.95%) respectively at 6 months. DNA chimerism was confirmed by qPCR. At this level of microchimerism, there was no difference in haematological parameters between untreated and injected Hbbth3/+ progeny.

In contrast to cell therapy, haematological indices in Hbbth3/+ recipients showed marked improvement with lentiviral treatment over controls from 4 to 20 postnatal weeks (n=2). Red cell counts increased to 8.3+/-1.2 E+6/µl (vs. 5.2+/-0.2E+6/µl, $p<0.05$) and haemoglobin levels improved to 12+/-1.4g/dL (vs. 9.6+/-0.3g/dL, $p<0.05$). Despite this, blood smears showed
persistent evidence of haemolysis. Vector copy number in peripheral blood ranged from 0.2-4.6 (median 2.4 copies per cell).
ConclusionIUHSCT at current cell doses resulted in microchimerism with no significant haematological improvement. In contrast, lentiviral IUGT showed greater efficacy in treating Hbbth3/+ mice, although we observed only partial albeit significant correction. Despite this initial success, the long-term effects of LV use must be determined as there are numerous safety concerns. As the safety profile of IUHSCT is likely to be more favourable, we are currently studying various strategies to improve engraftment, including postnatal cell boosting.

W-1181

IMMUNOSUPRESSION MEDIATED BY HUMAN ADIPOSE DERIVED MSC INVOLVES A CELL CONTACT-DEPENDENT MECHANISM.

Suzdaltseva Yulia, Goryunov Kirill, Rubtsov Yuri, Tkachuk Vsevolod
Moscow State University, Moscow, Russian Federation

Human adipose-derived mesenchymal stem cells (hASCs) are the candidate cellular therapeutics in regenerative medicine. Extensive research has focused upon the MSC-dependent immunomodulate properties. Various molecular mechanisms can be involved in hASC-mediated immune suppression. These mechanisms underlying hASCs-mediated immunosupression include soluble factors and cell-cell contacts, which possibly depend on expression of cell adhesion molecules. In the present study the potential mechanism involved in regulatory cell induction by allogenic hASCs was examined. To dissect cell contact-dependent mechanism of hASC-dependent immune suppression in vitro co-culture system consisting of hASCs and phytohemaglutinin-activated peripheral blood lymphocytes (PBL) was established. hASCs isolated from adipose tissue were characterized for human MSC surface marker expression and differentiation potential. hASCs inhibited proliferation of activated PBL both in trans-well and cell-contact coculture system as assessed by CyQuant cell proliferation assay. hASC-conditioned growth medium had no effect on proliferation of activated PBL. Activation of lymphocytes with hASCs had also resulted in increased attachment of immune cells to hASCs. Flow cytometry analysis of bound CD4+ and CD8+ T cells, NK and B cells with fluorophore-conjugated antibodies against surface markers reveals that activated CD4+ T cells are capable to bind hASCs more effectively in comparison to non-activated. At the same time, the capability of other analyzed immune cells to bind hASCs decreased or did not change. The protein and mRNA levels of ICAM increased in both activated T-cells and hASCs in contact cocultures compared with cells cultured alone or separately in transwells. We showed that hASCs-dependent suppression of activated T-cell proliferation is not associated with reduction of IL-2R (CD25) expression or apoptosis. hASCs were shown to induce FoxP3 mRNA and protein expression in CD4+ T-cells. This effect required direct contact between hASCs and CD4+T-cells. On the other hand hASCs stimulation with activated lymphocytes induces indoleamine-2,3-dioxygenase (IDO) synthesis. The increased level of IDO mRNA was observed in hASCs co-cultured with activated PBMC in cell-cell contact system in comparison to separate cocultures. It addition, through use of antagonist and neutralizing antibodies, IDO were shown to correlate with induction CD4+CD25+FoxP3+ T-cells in cell-cell contact coculture. Taken together our data support the substantial role of cell contact-dependent mechanism in hASC-mediated immune suppression.

W-1182

SOMATIC PROGENITOR CELL HOMEOSTASIS IS DISRUPTED BY MITOCHONDRIAL DNA MUTAGENESIS IN PREMATURELY AGING MICE.

Kati J. Ahlqvist[1], Riikka H. Hämäläinen[1], Kirmo Wartiovaara[2], Tomas Prolla[3], Anu Suomalainen[1]

[A]Research Programs Unit, Molecular Neurology, [1]University of Helsinki, Helsinki, Finland, [2]Department of Genetics, University of Wisconsin, Madison, WI, United States

Mitochondria produce energy in form of ATP to be used in all cellular functions. Mitochondria have its own circular DNA encoding for 13 polypeptides, tRNAs and rRNAs. All the proteins encoded by the mitochondrial DNA (mtDNA) are subunits of respiratory chain complexes I, III, IV and V. ATP is synthesized by respiratory chain (RC), which also produces superoxide as a by-product, so RC defect may, besides decreasing ATP production, increase the production of reactive oxygen species (ROS). When mtDNA is mutated, it often leads to RC dysfunction, which in turn leads to ineffective energy production and/or increased ROS production.
Somatic stem cell (SSC) dysfunction is typical for different progeroid phenotypes in mice with genomic DNA repair defects. Increased mtDNA mutagenesis can also result in progeroid phenotype in mice. “Mutator” mice show extensive mtDNA mutagenesis because of an inactivated exonuclease function of the mitochondrial replicative DNA polymerase gamma (POLG). Their phenotype mimics premature aging, starting from 6-8 months of age, with progressive hair graying, alopecia, osteoporosis and general wasting. Their life span is limited to 13-15 months because of severe anemia, with age-dependent decline in erythro- and lymphopoiesis, recently suggested to be due to HSC dysfunction. POLG forms the minimal mtDNA replisome together with the mitochondrial single-stranded DNA binding protein and a replicative helicase, Twinkle. If dominant mutant Twinkle is overexpressed in mice, large-scale mtDNA deletions accumulate in postmitotic tissues (hence the “Deletor” mice), leading to progressive late-onset mitochondrial myopathy at 12 months of age and RC deficient neurons, but normal life span. We asked whether SSC dysfunction could contribute to the mtDNA mutagenesis-linked premature aging, and utilized the two mouse models with mtDNA maintenance defects, Mutator and Deletor, of which only the former showed a premature aging phenotype.
We found that Polg-Mutator mice had neural (NSC) and hematopoietic progenitor (HPC) dysfunction already from embryogenesis. NSCs extracted from E12 embryos showed accumulation of mtDNA point mutations and the self-renewal ability of NSCs was decreased in vitro. Also the amount of quiescent NSCs in adult brain was reduced in vivo.
Mutator fetal liver showed changes in erythropoiesis already before the onset of anemia. FACS analysis of fetal liver HPCs showed decreased amount of basophilic erythroblasts (CD71+,Ter119+), whereas the amount of more mature erythroblasts (Ter119+,CD71low) was increased. Mutator fetal liver HPCs produced more erythroid colonies compared to wild type cells when cultured in methylcellulose. N-acetyl-L-cysteine treatment rescued both NSC and HPC abnormalities during embryonal development, suggesting that subtle ROS/redox changes, induced by mtDNA mutagenesis, modulate SSC function. We also found delayed maturation of erythrocytes in adult Mutator peripheral blood, including ineffective clearance of transferrin receptor and organelles, and long-term NAC-treatment is ongoing.
Our results show that mtDNA mutagenesis affected SSC function early but manifested as respiratory chain deficiency in nondividing tissues in old age. Deletor mice, having mtDNA deletions only in postmitotic cells and no progeria, had normal SSCs. We propose that SSC compartment is sensitive to mtDNA mutagenesis, and that mitochondrial dysfunction in SSCs can underlie progeroid manifestations.

W-1183

RECRUITMENT OF HEMATOPOIETIC STEM AND PROGENITOR CELLS IS REGULATED BY THE ANGIOGENIC FACTOR EPIDERMAL GROWTH FACTOR-LIKE DOMAIN 7

Ismael Gritli, Chiemi Nishida, Aki Sato, Shinya Munakata, Hiroshi Shimazu, Yoshihiko Tashiro, Kaori Kusubata, Hiromitsu Nakauchi, Koichi Hattori, Beate Heissig
The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Epidermal growth factor-like domain protein 7 (Egfl7), known to play a role in angiogenesis, is expressed during embryogenesis, in hematopoietic stem cells (HSCs) as well as in certain myeloid leukemias. The contribution of Egfl7 to hematopoiesis has not yet been described. Herein, we studied the effects of forced expression of Egfl7 on murine hematopoiesis. Enforced expression of Egfl7 resulted in a marked enhanced myeloid and megakaryocytic cell

differentiation *in vivo*. Overexpression of Egfl7 expanded the number of hematopoietic stem and progenitor cells (HSPC) in the BM of non-myelosuppressed mice. In addition, adenoviral-mediated overexpression of Egfl7 into non-myelosuppressed mice caused mobilization of immature progenitors into circulation. Using a hematopoietic stress model, *Egfl7* was upregulated in BM cells upon Fluorouracil (5-FU)-induced injury. Overexpression of Egfl7 using an adenoviral delivery system resulted in accelerated hematopoietic recovery, especially of myeloid cells and platelets after 5-FU treatment, accompanied by a dramatic increase in HSPC within the BM. Overexpression of Egfl7 in mice accelerated the entrance of HSCs into cell cycle and myeloid (granulocytic) and megakaryocytic differentiation after myelosuppression. Kit-ligand, SDF-1α, and VEGF-A were identified as novel downstream target molecules after Egfl7-forced expression.
These data suggest that increased expression of Egfl7, as found after myelosuppression within BM cells or released from endothelial cells of a growing tumor, can promote myeloid cell proliferation and differentiation, and cause HSPC mobilization, most likely due to its ability to alter the secretion pattern of critical stem cell niche factors (Kit-ligand, SDF-1α and VEGF-A).

W-1184

COMPREHENSIVE ANALYSIS OF HEMATOPOIETIC STEM CELL NICHE

Aparna Venkatraman, Xi C. He, Ariel Paulsonl, Hua Li, Linheng Li, Ryohichi Sugimura Sugimura
Stowers institute for medical research, Kansas city, MO, United States

Bone marrow niches and the signals emanating from them are critical for maintaining quiescent and active hematopoietic stem cells (HSC). Though a variety of stromal cells are reported to be HSC niche components, the integration of molecular signals emanating from different niche component to maintain different states of HSC is largely unclear. Combining high throughput RNA sequencing with protein expression analysis, we provide a comprehensive view of the unique and combinatorial ability of several niche components to maintain various HSC states. The reporter mice used were Nestin+, Cspg4+, Sdf+(mesenchymal stromal cells), Col2.3+(mature osteoblasts, mOB),Col3.6+, N-Cadherin+ (pre-osteoblast, pOB) and Pecam+(endothelial cells). Megakaryocytes, macrophages and HSCs were separated using surface markers. Analysis of major signaling pathways in stem cell function revealed at least three distinct zones.
The Endosteal zone- This zone mainly comprises of mOBs (Col2.3+) and pOBs (N-Cadherin+). Our data revealed that the two types of cells are quite distinct. Genes involved in maintenance of HSC quiescence including Opn, Cxcl12 and Angpt2 were preferentially expressed in N-cadherin+ pOBs. Members of BMP signaling like BMP4 known to play key role in stem cell quiescence were preferentially expressed in N-Cadherin+ pOBs. Analysis of Wnt signaling revealed members of non-canonical Wnt signaling and inhibitors of canonical Wnt signaling were predominantly expressed in N cadherin+ cells. Cytokines were minimally expressed in this zone. Cell adhesion molecules of cadherin family- Cdh2, 11; Integrin family- Itga 7, 8 and 11 were uniquely expressed in N cadherin+ cells.
The Perivascular zone- This mainly comprised of stromal cells around the arteriole and included Nestin+ and Cspg4+cells. Unique expression in Nestin+ included BMP signaling antagonist Noggin and Lif , members of both canonical and non-canonical Wnt signaling. Unlike the endosteal zone, expression of canonical Wnt signaling inhibitors was negligible. Cytokines and cell adhesion molecules were less abundant in this zone.
The Sinusoidal zone- This comprised of Pecam+ cells and megakaryocytes since sinusoids and these differentiated hematopoietic cells are present scattered throughout the central bone marrow. Ligands of Notch signaling, including Jag1, 2, and Dll4 were uniquely expressed in Pecam+ stromal cells. Jagged 1, involved in retention of HSC in the undifferentiated state, was also expressed in N-Cadherin+ pOBs. SCF as ligand for cKit were preferentially expressed in Pecam+ stromal cells. Cell adhesion molecules of the integrin family and members of Fgf signaling involved in HSC proliferation, homing and mobilization were preferentially expressed in this zone.
In summary, our data indicates that N-cadherin+ cells present in the endosteal zone have the highest expression of genes involved in stem cell quiescence. In contrast, suppressed BMP signaling, and presence of canonical and non-canonical signaling members in Nestin+ cell in peri-vascular zone suggested HSCs in this zone are in a binary state of quiescence and active. High expression of Scf, Fgfs, and Notch ligands in Pecam+ cells and megakaryocytes suggest that the sinusoidal zone support proliferation and mobilization of stem cells. Hence this comprehensive analysis in

gene and protein reveal that different niche components play unique as well as co-operative roles for maintenance and regulation of different states of HSCs.

W-1185

DELETION OF NOTCH1 TRANSCRIPTIONAL ACTIVATION DOMAIN REVEALS A NOVEL ROLE FOR NOTCH1 SIGNALING IN FETAL HEMATOPOIETIC STEM CELLS

Kostandin V. Pajcini[1], Dawson Gerhardt[1], Teresa D'Altri[4], Lanwei Xu[1], Olga Shestova[2], Jonathan Epstein[3], Nancy Speck[3], Anna Bigas[4], Warren S. Pear[1]

[A]Pathology and Laboratory Medicine, [B]AFCRI Cancer Institute, [C]Cell and Developmental Biology, [1]University of Pennsylvania, Philadelphia, PA, United States, [2]Cancer Research Program, IMIM-Hospital del Mar, Barcelona, Spain

Notch1 contains a conserved transcriptional activation domain (TAD) that is required for optimal signaling in vitro and for Notch1-induced T-ALL. To determine its developmental function, knock-in mice lacking the Notch1 TAD (ΔTAD/ΔTAD) were generated. Unlike Notch1 knockouts, ΔTAD/ΔTAD mice survive until late gestation, succumbing to multiple cardiac abnormalities. Notch1 is required to generate the earliest embryonic hematopoietic stem cells (HSC); however since Notch1-null embryos die early in gestation, additional functions for Notch in embryonic HSC biology have not been described. Notch1 TAD deficient HSCs successfully transit to the E11.5 fetal liver, but are decreased in frequency compared to wild type and are defective in competitive bone marrow transplant experiments, a phenotype recapitulated in RBPJf/f conditional knockout mice. Loss of Notch1 TAD impairs Notch1 core complex formation on the Hes1 promoter and consequently inhibits Hes1 expression. Together, these studies reveal an essential role for the Notch1 TAD in embryonic development and identify important cell autonomous functions for Notch1 signaling in fetal HSC homeostasis.

W-1186

HUMAN FETAL BONE MARROW MESENCHYMAL STEM CELL DERIVED FROM SECOND-TRIMESTER FETUS DEMONSTRATE A STRONGER HEMATOPOIETIC SUPPORTIVE CAPACITY THAN FETAL LIVER MESENCHYMAL STEM CELL

Xiao Hu[1], Yongjuan Duan[1], Fang Li[2], Yi Sun[1], Chunmin Fang[1], Xiaoting Wang[1]

[1]Institute of Hematology, The Chinese Academy of Medical Sciences, Tianjin, China, [2]Shandong University, Jinan, China

Mesenchymal stem cells, which play a major role in establishing hematopoietic microenvironment, have been isolated from various tissues in the early developing embryo and the adult organism. During ontogeny, hematopoietic stem cells (HSC) shift from fetal liver and spleen to adult bone marrow and this movement may be due to favorable inductive signals from different microenvironments. It is of great interest for both basic research and translational application to understand the nature of the hematopoietic microenvironment. A few *in vitro* study has demonstrated that fetal bone marrow MSC(FBM-MSC), compared with adult bone marrow MSC(ABM-MSC), is less perfect for adult HSC maintenance and differentiation. However, similar evidence is lacked for the fetal HSC in fetal liver MSC (FL-MSC) and fetal bone marrow MSC (FBM-MSC) which represents the fetal hematopoiesis. This study was aimed to compare the hematopoietic supportive capacity for fetal HSC and adult HSC between fetal liver and fetal bone marrow MSC *in vitro*.

Fetal liver MSC (FL-MSC) and fetal bone marrow MSC (FBM-MSC) of the same fetus were isolated from three second trimester fetuses between gestations of 16 to 25 weeks. Another six fetal liver MSCs were isolated from gestation stage matched and unmatched fetuses. After irradiation, MSC were co-cultured with CD34(+) cells isolated from umbilical cord blood (UCB-CD34+)or fetal liver (FL-CD34+). The total nucleated cell number were counted and the phenotypic changes of co-cultured CD34(+) cells were analyzed by flow cytometry and the colony forming unit cells were analyzed by CFU forming assay. UCB-CD34+cells were drive into erythroid differentiation by cytokine

cocktail then co-cultured with FL-MSC or FBM-MSC for terminal maturation and enucleation. Erythroid culture was analyzed by flow cytometry with CD71 and CD235a antibodies and DNA dye LDS751.
The results showed that cell morphology varies between FL-MSCs and FBM-MSCs, yet immunophenotype of CD34, CD45, CD105, CD49e, CD90, CD44 expression are comparable between FL-MSCs and FBM-MSCs. Both types of cells demonstrated similar osteoblastic differentiation capacity, yet FL-MSC has less strong adipogenic differentiation capacity. FBM-MSC had a stronger hematopoietic supportive capacity than FL-MSC for both fetal and adult HSCs, yet the supportive capacity is more prominant for adult HSCs than fetal HSCs. In addition, FBM-MSC has a stronger capacity to promote terminal maturation of UCB-CD34+ erythroid culture. Both of them enhanced the differentiation of CD34(+) cells into myeloid lineages. The study also found that cytokines were expressed differently in FBM-MSC and FL-MSC. The study supports the hypothesis that hematopoietic stem cells move to favorable microenvironment during ontogeny and provides clues to engineer better hematopoietic niches for *in vitro* hematopoietic differentiation.

Endothelial Cells/Hemangioblasts

W-1191

HIGHLY EFFICIENT AND SCALABLE GENERATION OF FUNCTIONAL ENDOTHELIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS BY A NOVEL GSK3 INHIBITOR.

Christoph Patsch[1], Eduard Urich[1], Eva Christina Thoma[1], Klaus Christensen[1], Tobias Heckel[1], Lin Sun[2], Xin Jiang[2], Wei He[1], Per-Ola Freskård[1], Paul Huang[3], Dorothee Kling[1], Martin Graf[1], Roberto Iacone[1]
[1]Pharma Research Early Development, Roche, Basel, Switzerland, [2]Harvard Stem Cell Institute, Cambridge, MA, United States, [3]Massachusetts General Hospital and Harvard Medical School, Cardiovascular Research Center and Cardiology Division, Boston, MA, United States

The use of human pluripotent stem cells (PSCs) for *in vitro* disease-modeling and clinical applications is limited by the ability to rapidly produce pure populations of various cell types in sufficient quantities.
Herein, we describe a scalable monolayer protocol to induce vascular cells in chemically defined conditions driven by a novel and potent GSK3ß inhibitor. Within six days we generated large cell populations that are highly enriched for endothelial cells (VE-Cadherin$^+$ ≥85%). We isolated VE-cadherin$^+$ cells by magnetic activated cell sorting (MACS) to ensure pure and homogenous endothelial cell cultures (≥98%). Time-resolved whole-genome expression and selective qRT-PCR analysis revealed a gene expression pattern closely resembling early embryonic vasculogenesis. Further characterizations of VE-Cadherin$^+$ cells, confirm a functional endothelial phenotype. Stem cell-derived endothelial cells give rise to continuous endothelium with dynamic barrier function properties, form vascular network-like structures in angiogenesis assays, show *in vivo* angiogenic potential, convert into activated endothelium after treatment with pro-inflammatory cytokines, thereby facilitating the recruitment of leukocytes. Overall the purified VE-Cadherin$^+$ cells present an endothelial-specific expression pattern; positive for PECAM-1$^+$, CD34$^+$, VE-Cadherin$^+$, vWF$^+$, CXCR4$^+$, VEGFR2$^+$, VEGFR3$^+$ and negative for the hematopoetic lineage markers CD45$^-$, CD43$^-$ and for the smooth muscle cell markers PDGFRβ$^-$, SMA$^-$ The endothelial cell population maintained their cellular identity over the period of cultivation.
This novel and robust method allowed us to reproducibly generated large numbers of homogenous endothelial cells from more than 20 different PSC lines including disease-specific lines from patients that have vascular complication associated with Type 2 Diabetes. With the growing need for defined protocols, our differentiation system may become the standard for deriving endothelial cells at relevant scales appropriate for drug discovery campaigns and regenerative therapies.

W-1192

NEUROPILIN-1 IDENTIFIES THE EMERGENCE OF ENDOTHELIAL COLONY FORMING CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Nutan Prasain[1], Man R. Lee[2], J. Luke Meador[2], Sasidhar Vemula[1], Momoko Yoshimoto[1], Michael J. Ferkowicz[1], Hal E. Broxmeyer[2], Mervin C. Yoder[1]

[A]Pediatrics, [1]Indiana University School of Medicine, Indianapolis, IN, United States

Endothelial colony forming cells (ECFCs) are rare circulating endothelial progenitor cells with robust clonal proliferative potential that display intrinsic *in vivo* vessel forming ability. While ECFCs form durable and functional blood vessels *in vivo*, they are rare in number in peripheral blood and their number tends to decline with age and disease. Thus, harnessing autologous ECFCs derived from patient specific induced pluripotent stem (hiPS) cells may be an innovative alternative approach to repair the damaged blood vessels in patients with peripheral artery disease (PAD) at risk for critical limb ischemia. While directed differentiation of hiPS and human embryonic stem (hES) cells into the endothelial lineage has been reported to generate endothelial cells with a mature endothelial phenotype possessing low proliferative potential, derivation of endothelial cells with ECFC properties (progenitor cells with clonal proliferative potential and *in vivo* vessel forming ability) from human pluripotent stem (hPS) cells (both hiPS and hES cells) has not yet been reported.

In order to identify ECFCs from hPS cells undergoing endothelia lineage differentiation, we utilized previously reported protocols (both mouse stromal OP9 cells and embroid body-induced endothelial differentiation protocols) to differentiate hiPS and hES cells into endothelial lineages and characterized isolated putative endothelial cells for ECFC properties. The putative endothelial cells that were derived from these protocols exhibited low clonal proliferative potential and were highly unstable often transitioning into non-endothelial phenotype and failed to give rise to functional in vivo vessels upon implantation into immunodeficient mice, suggesting that endothelial cells derived from the existing protocols lacked the ECFC properties. Therefore, based upon the known importance of vascular endothelial growth factor (VEGF) signaling pathway in the emergence of endothelial cells during development, we developed a novel endothelial differentiation protocol and found neuropilin-1 (NRP-1), a VEGF co-receptor, as an early marker for identifying the emergence of ECFCs from hPS cells. Endothelial lineage differentiation was initiated by growing hPS cells in endothelial differentiation media supplemented with growth factors for 12 days on Matrigel coated dishes. On day 12, cells were harvested and sorted using antibodies that recognize endothelial antigens CD31, CD144, CD146, and NRP-1. Sorted cells were subsequently cultured in endothelial growth (EGM-2) media. Compared to other sub-sets of sorted cells, only NRP-$1^{+}$$CD31^{+}$ cells exhibited ECFC properties. NRP-$1^{+}$$CD31^{+}$ formed a homogenous monolayer with characteristic cobblestone appearance, exhibited clonal proliferative potential, demonstrated angiogenic behavior by forming capillary like structures when cultured on Matrigel, and formed robust *in vivo* vessels when in implanted in immune deficient mice. Collectively, we conclude that the emergence of ECFCs from hPS cells is identified by the endothelial subset expressing NRP-1. Overall, these studies would provide new information on a potentially critical factor and mechanism that control specification and emergence of endothelial cells with ECFC properties from hiPS cells, thereby, provide an innovative strategy to produce large numbers of autologous *in vivo* vessel forming cells to restore deficient microvasculature in patients with cardiovascular disease.

W-1193

A SYSTEMATIC APPROACH TO ELUCIDATING THE OPTIMAL CONDITIONS FOR EFFECTIVE HARVEST OF ENDOTHELIAL OUTGROWTH CELLS

Dedy Sandikin[1], **Mark Chong**[2], Mahesh A. Choolani[1], Jerry K Y Chan[3]

[A]Obstetrics and Gynaecology, [1]National Univ of Singapore, Singapore, Singapore, [2]Reproductive Medicine, KK Women's and Children's Hospital, Singapore, Singapore

Endothelial Progenitor Cells (EPC) from circulating blood have been the subject of intense research for diagnostic and therapeutic applications. In particular, Endothelial Outgrowth Cells (EOC), an EPC subset, demonstrate lineage stability and proliferation capacity, leading to their proposed use in tissue engineering and regenerative medicine. However, translation to clinics has been hampered by significant differences in experimental observations across research groups, leading to questions on reproducibility of results and, indeed, on the exact identity of these cells.

We hypothesised culture conditions to influence harvest efficiency of EOC and sought to optimise the protocol for maximal EOC yield from umbilical cord blood (UCB). Following informed consent, term UCB (n=86) were obtained and the influence of mononuclear cell (MNC) separation, seeding density, sera and substrates were systematically evaluated. In spite of a 10-fold reduction in total nucleated cell counts following density centrifugation, EOC could not be generated from direct cultures of whole blood, suggesting the process of red blood cell depletion to be critical. Subsequently, seeding density was studied and established to be inversely related to harvest yield, with a seven-fold increase in number of EOC colonies obtained following a four-fold reduction in seeding density ($p<0.01$). The effects of sera were then studied. Varying serum concentrations from 10 to 50 percent of total culture medium was not found to significantly influence yield. Strikingly, batch-dependence was found to be the major factor, with EOC found to be reliably generated from only two out of seven lots of sera tested from three companies. Finally, substrate coating was not found to influence the number of colonies generated, nor cellular phenotype as assessed by surface marker profile and functionality, as assessed by Matrigel network formation assays. In conclusion, the isolation of EOC from UCB was successfully optimised, with recommendations for red blood depletion, optimal seeding density and batch testing of sera for the reproducible generation of EOC. Identification of sera components that promote or inhibiting colony formation is currently under way.

W-1194

DOSAGE OF VASCULAR ENDOTHELIAL GROWTH FACTOR REGULATES HEMATOPOIETIC STEM VERSUS PROGENITOR CELL FATE IN HEMOGENIC ENDOTHELIUM

Lydia K. Lee, Masaya Ueno, Luisa Iruela-Arispe, Hanna A. Mikkola
Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, Los Angeles, CA, United States

Healthy donors comprise the only source of transplantable hematopoietic stem cells (HSC) to date, as their production *in vitro* from pluripotent cells has been unsuccessful. So far, the mechanisms that dictate hemogenic endothelium fate to multipotent, self-renewing HSC rather than transient myeloerythoid progenitors are poorly understood. A critical signaling pathway acting on embryonic mesoderm and endothelium is the Vascular Endothelial Growth Factor A (VEGF-A)/VEGFR2 pathway, a potent regulator of vasculogenesis and angiogenesis. Although its function in embryonic hematopoiesis has been implied, developmental studies using *in vivo* mammalian models are scarce due to the lethality of the *VEGF* heterozygous embryos. To overcome this hurdle and obtain germline heterozygous mutants, we intercrossed female *Vasa-Cre* transgenic mice with $VEGF^{fl/wt}$ males. Remodeling of the yolk sac and embryo vasculature into mature branched networks was severely impaired in haploinsufficient concepti, which never developed beyond E10.5. Surprisingly, although the yolk sac is the site of emergence for the first hematopoietic wave of “primitive” erythroid cells as well as the second wave of “transient-definitive” myeloerythroid restricted progenitor cells, its potential to form either primitive erythroid or myeloerythroid colonies in methylcellulose was unaffected. This finding contrasted prior reports of a major defect in the generation of primitive red blood cells (RBC). FACS analysis and immunofluorescence (IF) confirmed the presence of Ter119+CD71+ βH1-globin primitive RBCs in the yolk sac and the placenta, and suggested that improper vascularization, rather than perturbed hematopoiesis, was the cause for the pale yolk sac.
In contrast, fewer mixed myeloerythroid colonies developed from explants of the placenta and the caudal half of the embryo, which are sites for the final and “long-term definitive” wave generating the multipotent HSCs that differentiate into myeloid, erythroid as well as lymphoid lineages. Moreover, hematopoietic organs from heterozygous embryos could not grow either B or T-lymphoid cells in OP9 and OP9-DL1 stroma co-culture, respectively. Furthermore, IF revealed reduced arterialization in VEGF-A-deficient placenta. These data suggest that proper dosage of VEGF-A is required to develop HSCs and establish arterial identity that is necessary for the specification of hemogenic endothelium to HSC fate.
To define the critical sources of VEGF-A during hematopoiesis, we used complementary tissue-specific genetic models of *VEGF-A* inactivation. Deletion of one *VEGF-A* allele from Tie2-expressing endothelial cells generated mutant embryos that were indistinguishable from their control littermates and robustly generated all types of hematopoietic stem/progenitor cells. Similarly, deletion of one allele of *VEGF-A* from Mesp1-expressing mesodermal cells in

embryonic hemogenic tissues and the heart did not abolish the generation of pRBCs, myeloerythroid progenitors or lymphoid cells. Finally, we deleted one or both *VEGF-A* alleles exclusively from the placental trophoblasts by infecting the trophectoderm with a lentiviral vector encoding Cre. No mutants survived beyond the 26 somite-pair stage, and yolk sac vasculature was poorly remodeled. These data suggest that placental trophoblasts constitute a vital source of VEGF-A during development that may even support hematopoiesis and vasculogenesis in distant hemogenic tissues.

W-1195

INVESTIGATING THE DIFFERENTIATION OF ARTERIAL AND VENOUS SUBPOPULATIONS OF HUMAN EMBRYONIC STEM CELL DERIVED ENDOTHELIAL CELLS - APPROACH TO VESSEL GENERATION

Edit Gara, Judit Skopál, Annamária Kosztin, Zsolt Bagyura, Tamás Németh, Béla Merkely, Gábor Földes
Heart Center, Semmelweis University, Budapest, Hungary

Human pluripotent stem cell derivatives offer the promise for novel therapeutic possibilities in cardiovascular disease. In this study, we aimed to identify signals responsible for induction of arterial and venous endothelial differentiation. Undifferentiated H7 human embryonic stem cells (hESC) were differentiated into endothelial lineage using monolayer and embryoid-body formation methods. The CD31-positive cells were separated from the differentiating culture by FACS. Human ESC-derived endothelial cells (hESC-ECs) were characterized by their morphological, immunocytochemical and in vitro functional properties. Cells were stained positive for panendothelial markers CD31, von Willebrand factor, and ve-cadherin by immunocytochemistry; cells form capillary-like tubules on Matrigel and can take up acetylated-low density lipoprotein. The role of hypoxia (5% O2) and vascular endothelial growth factor (VEGF) supplementation were assessed on the differentiation of arterial and venous subpopulations. We showed that both hypoxia and VEGF tended to improve the differentiation yield (control, 0.2%; hypoxia, 0.3%; VEGF, 0.7%), however, it stayed low. As assessed by quantitative PCR, culture of hESC-EC abundantly expressed both arterial (EphrinB2, Notch1-2) and venous (EphB4) endothelial markers. The mRNA of lymphatic-specific marker, FLT4 was not detectable. VEGF markedly increased the mRNA levels of arterial endothelial genes as well as venous marker EphB4 in the differentiating culture (Notch1, 18.89±3.75; Notch2, 9.01±2.97; EphrinB2, 11.89±5.29; EphB4, 10.10±3.04-fold increases vs. hESC, all $p<0.01$, n=3). Hypoxia induced higher endothelial marker expressions in monolayer than in EB-derived cultures (Hypoxia-method: Notch1, 6.01±1.68; Notch2, 6.46±1.50; EphrinB2, 2.93±0.68 - fold changes vs hESC all $p<0.05$; EphB4 3.72±1.01 - fold increase vs. hESC; EB-method: Notch1, 1.15±0.60; Notch 2 0.60±0.14; EphrinB2, 0.77±0.13; EphB4, 0.70±0.15 - fold increase vs. hESC,n=3).To test in vivo viability and maturation of the cells, we have transplanted hESC-EC and control HUVEC subcutaneously into athymic nude rats. Immunohystochemistry has proven that hESC-EC form capillary-like structures in vivo. After implantation, the mRNA levels of arterial and angiogenesis markers were significantly higher as compared with preimplanted cells (fold increases, mean±sem: angiopoietin2, 86.23±26.34; apelin, 1197±703, Notch1 440±303; EphrinB2 58±37; n=5, $p<0.05$). Endothelial derivatives of human pluripotent stem cells can be promising future options for tissue engineering in ischaemic cardiovascular diseases and vascular regeneration. VEGF and hypoxia may act as key extracellular signals in the differentiation process toward more specific endothelial subpopulations.

W-1196

HUMAN ENDOTHELIAL COLONY FORMING CELLS (ECFCS) - IMMUNOGENICITY STUDIES UNDER INFLAMMATORY CONDITIONS AND THE PRESENCE OF MESENCHYMAL STROMAL CELLS (MSCS)

Naima Souidi[1], Katharina Schallmoser[2], Dirk Strunk[2], Caroline Schmidt-Lucke[3], Hans-Dieter Volk[1], Martina Seifert[1]
[1]Immunology, Berlin-Brandenburg Center for Regenerative Therapies / Charité, Berlin, Germany, [2]Hematology/ Stem Cell Transplantation, Medical University of Graz, Graz, Austria, [3]Cardiology/Pneumology, Charité - Universitätsmedizin, Berlin, Germany

Introduction: Endothelial Colony Forming Cells (ECFCs) have been described to have regenerative properties by enhancing endogenous vascular repair processes and forming vascular networks in vitro as well as in vivo. To consider

ECFCs for therapeutic purposes, the cells must be analyzed regarding their immunogenic capacity in an inflammatory environment. Potential therapeutic co-application with Mesenchymal Stromal Cells (MSCs) might not only stabilize vessel formation, but could also promote modulation of ECFC immunogenicity in an inflammatory surrounding.
Methods: We isolated pairs of human umbilical cord derived ECFCs and MSCs from healthy donors and checked for phenotypic and immunogenic marker expression and differentiation capacity. Both cell types were exposed to different pro-inflammatory cytokines for 24h and stained for HLA- and adhesion molecules. Induction of CD4+ T cell-proliferation by ECFCs and MSCs was analyzed using a CFSE-based proliferation assay and measured by multicolor flow cytometry. To assess the potential immunomodulatory cross-talk between both cell types, we additionally performed proliferation assays with mitogen-triggered CFSE-labeled peripheral blood mononuclear cells (PBMCs).
Results: ECFCs and MSCs showed strong constitutive expression of HLA-ABC and ICAM-1. After stimulation with different pro-inflammatory cytokines (TNFα, IL-1β and IFNγ) for 24 hours, both cell types showed enhanced expression of both surface markers. ECFCs triggered with TNFα and IL-1β upregulated the adhesion molecule VCAM-1, whereas MSCs did not respond to IL-1β stimulation. Exposure to IFNγ dose-dependently induced expression of HLA-DR in ECFCs after 24h. In contrast, MSCs remained negative for HLA-DR during the same time frame but showed a delayed upregulation after three days.
The co-culture of IFNγ pre-treated ECFCs with CD4+T cells induced some immune cell proliferation. In contrast, unstimulated as well as IFNγ-treated MSCs failed to trigger proliferation responses. When tested in mitogen-stimulated assays, only MSCs were able to reduce PBMC proliferation significantly. Co-application of ECFCs and MSCs diminished the MSC effects.
Conclusion: ECFCs, in contrast to MSCs, show restricted immunogenicity via the up-regulation of HLA-DR by IFNγ. MSCs were not able to influence the immunogeneic potential of ECFCs. However, ECFCs could reduce the immunomodulatory effects of MSCs.

W-1197

A HIGH-THROUGHPUT SCREENING TO STUDY THE ROLE OF THE HEMANGIOMA-DERIVED STEM CELL (HEMSC) IN TUMOR FORMATION

Shaghayegh Harbi
New York University, New York, NY, United States

Infantile hemangioma (IH) is the most common tumor of infancy. However, little is known of its etiology and pathogenesis of infantile hemangioma. The overall goal of this investigation is to understand the process of hemangiomagenesis – defined as human vascular differentiation, followed by vascular regression and adipogenesis – by characterizing the progenitor hemangioma-derived stem cells (HemSCs). To understand the tumorigenic potential of HemSCs at the single cell level, genetic signatures and cell surface markers need to be identified via a multifaceted and targeted approach, using fluorescence-activated cell sorting (FACS)-purified/enriched **HemSCs** to highlight expression signatures between the progenitor – HemSCs and its derivatives.
Based on the current knowledge of IH biology, we hypothesize that the hemangioma-derived stem cell (HemSC) is a vascular stem/progenitor cell whose proliferation is dysregulated but not fully transformed, that orchestrates hemangioma pathophysiology via sophisticated activation of multiple signaling and regulatory networks.
To explore this hypothesis we propose to develop the following Specific Aims: 1) **To perform a high-throughput FACS analysis of cell surface markers and confirm the results by fluorescence bioimaging:** Through a high throughput screen, characterize human cell surface markers (242-antibody screening) to differentiate between **tumor biomarkers** (diagnostic tool) and **tumor stem cell biomarkers** (therapeutic tool). 2) **To characterize the gene expression profiles and patterns:** Characterize the gene expression profiles and patterns by microarray analysis followed by qRT-PCR and high-throughput analysis using lab-on-a-chip approach with single cell microfluidic analysis (microfluidic based single cell transcriptional analysis and fluorescence-activated cell sorting) of the cultured (FACS sorted) HemSCs and endothelial cells (human umbilical vein endothelial cells, HUVEC); HemSCs and the heterogeneous cell population following exposure to dexamethasone (the steroid commonly used for clinical management

of IH); HemSCs and glioblastoma CD133+ stem cells; HemSCs and Mesenchymal/Bone Marrow/Cord Blood CD133+ stem cells.

Significance Identifying a stem cell (HemSC) as a cellular precursor - with the ability to self renew and give rise to derivative cell types (with a differentiation potential) - two key functional properties of adult stem cells, is paramount in disease intervention and direct drug target therapy of IH as well as in regenerative medicine. Thus further identification and characterization of the HemSCs will lead to practical implications for clinical application. Reaching this goal requires advanced technology for *quantitative characterization of the FACS enriched HemSCs to characterize the cellular biomarkers and the gene expression profiles of the single HemSC.* This approach will provide distinct gene set signatures for diagnostic assays and drug targeting. Understanding the vasculogenic mechanism that controls IH development can also afford understanding the mechanisms that mediate the transformation of certain cancer stem cells into tumor endothelial cells - such as the differentiation of glioblastoma CD133+ cells into endothelial progenitors - potentially indicating a therapeutic target for treating proliferating hemangiomas.

W-1198

RSK4 SUPPRESSES ENDOTHELIAL CELL FATE THROUGH DIRECT INHIBITION OF PKA ACTIVITY

Taichi Matsunaga[1], Kohei Yamamizu[1], Kazuko Koshiba Takeuchi[2], Mizuyo Kojima[2], Jun K. Takeuchi[2], Jun K. Yamashita[1]

[1]Department of Cell Growth & Differentiation, Center for iPS Cell Research and Application, KYOTO UNIVERSITY, Kyoto, Japan, [2]Division of Cardiovascular Regeneration, Research Center for Epigenetic Disease,, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan

Endothelial cell (EC) differentiation is strictly regulated to generate functional blood vessel. To elucidate the underlying mechanisms of EC differentiation, we have previously established in vitro differentiation system and demonstrated that fetal liver kinase 1 (Flk1) + mesodermal cells derived from ES cells serve as common progenitor of both EC and Mural cells (MC; vascular smooth muscle cells and pericytes). We recently reported that cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) signaling enhanced EC differentiation from Flk1+ mesodermal cells via induction of VEGF receptors expression. We also revealed that expression of Ets variant2 (Etv2), a key molecule for hemato-endothelial fate, is triggered by PKA signaling. However, little is known about the regulation of PKA signaling in EC differentiation. To further dissect these sequential processes in vascular formation, we investigated a novel molecule target and its function during EC differentiation.

We carried out microarray analyses at various differentiation stages; undifferentiated ES cells, Flk1+ mesodermal cells to ECs and MCs. Then, we found that isoforms of p90 ribosomal S6 protein kinase (RSK) family were specifically expressed in differentiation stages. RSK1 was predominantly expressed in undifferentiated ES cell. RSK2 and RSK3 were highly expressed in EC. RSK4 was expressed in Flk1+ mesodermal cells and diminished in ECs. RSKs are mediators of the Ras/mitogen-activated protein kinase (MAPK) signaling cascade, which controls various biological functions, including cell growth, proliferation and survival. However, the role of RSK family that is still uncharacterized during EC differentiation.

To investigate roles of RSKs in EC differentiation, we examined the effect of a broad-spectrum RSKs inhibitor, SL0101, in our system. SL0101 treatment enhanced VEGF receptor expression and EC differentiation specifically with the simultaneous cAMP signal. SL0101-elicited EC differentiation was abolished by knockdown of PKA catalytic subunit alpha (PKAc), suggesting that the regulation by RSK during EC differentiation is depended on a PKA pathway. To identify RSK isoform that regulates EC differentiation, we investigated EC differentiation using shRNA against each RSK isoform. Among RSK families, RSK1-3, RSK4 knockdown in Flk1+ mesodermal cells similarly showed an increase of EC differentiation via induction of Flk1 and Etv2 expression even in the absence of SL0101. To demonstrate whether RSK4 interacts to PKA, we investigated the mechanisms linking PKA activity to RSK4. The immunoprecipitation and immunoblotting experiments indicated that RSK4 has potential to bind to PKAc. Moreover, RSK4 overexpression significantly decreased PKA activity. Furthermore, to investigate the biological function of RSK4 in vivo, we performed ex vivo whole embryo culture assay. Embryos that were cultured in the presence of SL0101

displayed an aberrant surface of yolk sac. Namely, SL0101 treatment dramatically enlarged the area of CD31+ vasculature in yolk sac.
Taken together, our findings are first evidences of strict system regulated by RSK4 to form correctly vascular development in mouse embryogenesis. RSK4 expression regulates endothelial cell fate through suppression of PKA-mediated Flk1 and Etv2 induction. Elucidation of the new-model cell fate determination by RSK4 would provide novel insights in developmental biology, stem cell biology, and regenerative medicine.

W-1201

HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS SYNERGIZE OSTEOGENIC/ODONTOGENIC DIFFERENTIATION OF PERIODONTAL LIGAMENT STEM CELLS IN 3D CELL SHEET

Chamila P. Panduwawala[1], Lakshman P. Samaranayake[2], Lijian Jin[3], Chengfei Zhang[1]
[A]Comprehensive Dental Care, [B]Oral Biosciences, [C]Periodontology and Public Health, [1]The University of Hong Kong, 34, Hospital Road, Sai Ying Pun, HK, Hong Kong

Objective:
To investigate the expression of odontogenic differentiation markers and vascular network formation in 3-D cell sheet with different cell ratios of periodontal ligament stem cells (PDLSCs) and human umbilical vein endothelial cells (HUVECs).
Method:
Human PDLSCs were isolated and characterized by flow-cytometry, and HUVECs were used for construction of cell sheet. Both types of cells were seeded on temperature responsive culture dishes with PDLSCs alone, HUVECs alone, and various ratios of these cells (1:1, 2:1, 5:1 and 1:5) to obtain confluent cell sheet layers. The expression of odontogenic pathway markers including ALP, BSP, RUNX2 were analyzed at 3 and 7 days using RT-PCR. Further ALP protein quantification was done at 7 and 14days using alkaline phosphatase assay. The calcium nodule formation was assessed qualitatively and quantitatively by Alizarin Red assay. Histological evaluations of three cell sheet constructs treated with different combinations (PDLSCs-PDLSCs-PDLSCs/PDLSCs-HUVECs-PDLSCs/Co-Culture 2:1 PDLSCs-HUVECs) were performed with H&E and immunofluorescence straining. Statistical analysis was done by t-test ($p<0.05$).
Result:
Significantly higher ALP gene expression was observed at 3days in 1:1 (PDLSCs:HUVECs) (2.52±0.67) and 5:1(4.05±1.07) co-culture groups compared with other groups($p<0.05$), being consistent with ALP protein quantification. However, BSP and RUNX2 genes expressions were higher at 7days compared to that detected at 3 days. Significant calcium mineralization was detected and quantified by Alizarin red assay at 14days in 1:1(1323.55±6.54µm) and 5:1(994.67±4.15µm) co-cultures as compared with mono-culture cell sheets($p<0.05$). H&E and CD31 immunostaining illustrated the development of a layered cell sheet structure with endothelial cell islands within the constructed PDLSCs-HUVECs-PDLSCs and co-culture groups. Furthermore, HUVECs permeated into layered cell sheet, was suggestive of rudimentary vascular network initiation.
Conclusion: This study suggests that PDLSCs-HUVECs co-culture models exhibit significantly high levels of odontogenic markers with signs of initial vascualar formation. This novel 3-D cell sheet-based approach may be potentially beneficial for periodontal regenerative therapy.

W-1202

ANGIOGENESIS IN PATIENTS WITH CHRONIC OCCLUSIVE ARTERIAL LOWER LIMBS DISEASE TREATED WITH AUTOLOGOUS STEM CELL AND EXERCISE

carlos franco[1], **Luz Mabel Avila Portillo**[2], Francisco Arroyo[1], Daniel Segura[1], Gonzalo Pullaz[2], Benjamin Ospino[2], Oscar Mauricio Alvarez[1], Jenniffer Priscila Avila[2]
[1]Hospital Militar Central, Bogota, Colombia, [2]Regenerative Medicine Stem Lab, Bogota, Colombia

Introduction:
Chronic occlusive arterial disease (COAD) affecting the lower limbs, is a disease resulting from atherosclerosis, also having the risk of progression to critical ischemia. Many
of these patients are not candidates for revascularization strategies; the stem cell transplantation becomes a viable treatment option.
.**Objectives:**
Evaluate the clinical response
obtained after autologous stem cell (ASCs) transplantation and a walking exercise program, in patients with chronic occlusive arterial disease of lower limbs without possibility of surgical revascularization.
Materials and
Methods: After signing informed
consent 24 patients with unilateral or bilateral COPD without possibility of revascularization (Fontaine II and III), were subjected to an ASCs intramuscular injection, obtained from peripheral blood or bone marrow, all the patients started therapy 3 times a week for 12 weeks at a Low Intensity 85% of maximum heart rate obtained during a stress test or formula 220-age x 85%. With the inclination and speed needed to induce claudication (moderate pain) in the first 3 to 5 minutes with duration of 30 minutes to 60 minutes treadmill with duration: 30 minutes to increase up to 60 minutes. At mode: Walk treadmill. In this study was evaluated the ankle / brachial index (ABI), pain visual analog scale (VAS) and free walking distance claudication (WDC) by comparing pre-transplant, two visits post-implementation and a year after the intervention.
Results:
The first evaluation was done by week 9.1 ± 3.3 and the second to 16.6 ± 5 days post injection. The third assessment was done at week 64.5 ± 4. All patients showed improvement in ABI I(0.52 ± 0.21 to 0.65 ± 0.13 p = 0.0023) and had decreased in the score by VAS of pain (from 9.1 ± 0.67 to 5.67 ± 1.3 p = 0.0000) compared with the first visit; difference increases when comparing the initial state with the second visit (0.52 ± 0.21 to 0.71 ± 0.14 p = 0.0001) for the ABI and VAS (from 9.1 ± 0.67 to 3.22 ± 2.4 p = 0.0000). The decrease in VAS for pain persisted over time even up to the assessment year (from 9.1 ± 0.67 to 2.22 ± 1.3 p = 0.0000). On the march there was a significant increase in distance traveled in meters after transplantation (from 86.2 ± 50.6 to 339.4 ± 119 p = 0.0000) of the significant between groups. There were no adverse effects or complications related to the procedure.
.**Conclusion:**
The autologous stem cells $CD34^{+}$ / $CD133^{+}$ through intramuscular injection plus a walking exercise
program is a clinically feasible and effective strategy to sustainable results in short and medium term, making it
, (viable) suitable tool for the
management of patients with severe lower limb atherosclerosis.

W-1203

UTILIZING STEM CELLS TO CREATE A NOVEL BLOOD-BRAIN BARRIER MODEL

Ethan Lippmann, Samira Azarin, Abraham Al-Ahmad, Jennifer Kay, Hannah Wilson, Sean Palecek, Eric Shusta

University of Wisconsin-Madison, Madison, WI, United States

The blood-brain barrier (BBB) is composed of brain microvascular endothelial cells (BMECs) which line brain capillaries and control trafficking between the brain and bloodstream via extremely restrictive intercellular junctions and a cohort of transporter proteins expressed by the endothelium. This unique interface is necessary to maintain brain health and homeostasis but presents a significant bottleneck for drug delivery: it excludes ~98% of small molecule therapeutics from reaching the brain. A long-standing goal in the research community has been the creation of a robust human *in vitro* BBB model to utilize for many purposes, including studies of BBB development and regulation and to use in high throughput screening of prospective neuropharmaceuticals. However, issues such as low yields, lack of scalability from primary sources, and poor barrier properties *in vitro* have hindered this field for several decades. To circumvent such problems, we have utilized human pluripotent stem cell (hPSC) technology to construct a high-fidelity BBB model. We developed a protocol for differentiating hPSCs to endothelial cells possessing characteristic BBB markers (GLUT-1, p-glycoprotein) and tight junction proteins (claudin-5, occludin). Derivation of these so-called hPSC-derived BMECs relies on the co-differentiation of neural cells that impart BBB characteristics on the developing endothelium in part via Wnt/β-catenin signaling. Purified hPSC-derived BMECs possess a substantial *in vitro* BBB phenotype, including elevated transendothelial electrical resistance (TEER) in response to co-culture with primary rat astrocytes (up to 1450±140 Ωxcm^2), selective permeability to small molecules of varying lipophilicity that correlates well with *in vivo* uptake, and expression of active efflux transporters. By incorporating other cells of the neurovascular unit into this BBB model, such as human pericytes derived from fetal tissue and human astrocyte/neuron mixtures derived from fetal neural progenitor cells, and optimizing medium conditions, we can further improve TEER up to 5000 Ωxcm^2. Overall, this model could have a variety of applications ranging from basic biological studies to screening candidate drugs for brain uptake.

W-1204

INDUCED HEMANGIOBLASTS DERIVED FROM EMBRYONIC STEM CELLS EFFECTIVELY IMPROVE VASCULAR REPAIR AND REGENERATION

Fang Liu[1], Suk Ho Bhang[2], Elizabeth Arentson[1], Chan Kyu Kim[1], Inyoung Kang[1], Jinsheng Yu[3], Judy Yoo[1], Nagisa Sakurai[1], Kyunghee Choi[1]

[A]Pathology & Immunology, [B]Department of Biomedical Engineering, [C]Department of Genetics, [1]Washington University in St. Louis, St. Louis, MO, United States

Generation of tissue restricted multipotent progenitors from pluripotent stem cells would be a powerful tool for regenerative applications. As for blood and vessel development, it has been shown that Flk-1+ mesoderm derived from embryonic stem (ES) cell or embryos contains the hemangioblast, a common progenitor of hematopoietic and endothelial cells. Our previous studies suggest that an Ets transcription factor, ER71/Etv2, can specify hemangioblast commitment from Flk-1+ mesoderm at the expense of cardiogenic Flk-1+ mesoderm generation. Herein, we reasoned that homogenous hemangioblast induction from ES cells could be achieved through cooperatively utilizing of defined hemangiogenic factors. By extensively screening of candidate genes preferentially expressed in the hemangioblasts, we were able to detect skewing outcome toward hemangiogenic (Flk-1+PDGFR-alpha-) mesoderm with Er71, Gata2 or Scl enforced expression. Furthermore, we demonstrated that ER71/Etv2, GATA2 and Scl form a core transcriptional network in hemangioblast development and that transient co-expression of these three factors during mesoderm formation stage in mouse ES cells robustly induced hemangioblasts by activating BMP and Flk-1 signaling and inhibiting PI3K and Wnt signaling. “The three factor-induced hemangioblasts” effectively generated hematopoietic and endothelial cells both in culture and in vivo. Importantly, mesenchymal stem cells (MSCs) protected induced hemangioblasts from apoptosis when co-cultured as spheroids and promoted their endothelial cell differentiation by producing VEGF and FGF2 angiogenic factors. Consequently, induced hemangioblasts, when co-delivered with MSCs as spheroids, generated functional endothelial cells and smooth muscle cells in ischemic mouse hindlimbs, resulting in improved blood perfusion and limb salvage. We provide a novel strategy to generate hemangioblasts and tissue spheroids of hemangioblast-MSCs for potential angiogenic repair and regeneration.

Other

W-2011

HIF1α EXPRESSION IN MULTIPLE ADULT STEM CELL COMPARTMENTS

Natsumi Takahashi, Juanita Mathews, Richard Allsopp
Institute for Biogenesis Research, University of Hawaii, Honolulu, HI, United States

Hypoxia inducible factor 1 alpha (HIF1α) is the principal transcriptional regulator in response to a low oxygen environment, activating a number of target genes for adaptive responses. Previously our group has identified HIF1α as a critical regulator of telomerase reverse transcriptase (Tert), the catalytic component of telomerase, in mouse embryonic stem cells. In the present study, we have begun to investigate the expression of HIF1α in mouse adult stem cell compartments. We observed low but detectable levels in resting c-Kit+Sca1+lineage^{Neg} Thy1.1^{Lo} hematopoietic stem cells (HSC), and readily detectable levels in CD133+ neuronal stem cells (NSC). Furthermore, immunohistochemical and western analysis revealed high levels expression of HIF1α in spermatogonial stem cells in both neonatal and adult testes as well as in both primordial follicles in neonatal ovary and mature oocytes in adult ovary. Exposure of both HSC and NSC to a hypoxic environment in vitro significantly induced Hif1 levels as well as Tert expression and telomerase activity ($P<0.01$). These observations point towards an important role of Hif1 in stem cell behavior, including regulation of telomerase.

W-2012

ASYMMETRIC AND SYMMETRIC REGULATION OF STEM CELL FATES BY HETEROCHRONIC AND WNT/MAPK PATHWAYS IN C. ELEGANS

Omid F. Harandi[1], Victor Ambros[2]
[1]*Medicine, Harvard Medical School/Beth Israel Deaconess Medical Center, Boston, MA, United States,* [2]*Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, United States*

Stem cells can switch between asymmetric (self-renewing) and symmetric (proliferative) cell division patterns and this transition must be tightly regulated for normal development and tissue homeostasis. Many genes that function in invertebrate asymmetric division have been shown to be deregulated in human malignancies and may act to promote proliferation of cancer stem cell by altering the balance between asymmetric and symmetric cell divisions. To genetically assess the role of pathways involved in transition from asymmetric to symmetric cell division, we utilized post-embryonic epithelial stem (seam) cells in *C. elegans* which permits us to study the genetic interactions of factors involved in temporal cell fate decision, self-renewal and polarity at the level of single stem cell *in vivo*. Here, we provide evidence that this transition is regulated by the activity of Wnt and MAPK pathways components, LIT-1/NLK (Nemo-Like Kinase) and POP-1 (TCF), and the temporal control of this transition is regulated by the heterochronic genes LIN-14, LIN-28, and *let-7-family* microRNAs. We propose that the heterochronic genes may modulate the temporal thresholds for LIT-1/NLK and POP-1/TCF activity in the stage-specific of these transitions by maintaining the polarity of APR-1/APC. Our results suggest a new emerging view of regulation of asymmetric to symmetric transition and polarity by heterochronic genes during normal stem cell development which might be deregulated in cancer stem cells.

W-2013

EFFECTS OF DIFFERENT SUBCULTURE TIMES ON CELL PROLIFERATION, REACTIVE OXYGEN SPECIES GENERATION AND APOPTOSIS OF FRESH OR CRYOPRESERVED EMBRYONIC STEM CELLS

Myungook Lee[1], Jung Ki Yoon[1], Jae Hee Lee[3], Seo Jin Oh[1], Ji Yeon Ahn[2], Jeong Mook Lim[1]
[A]*WCU Biomodulation,* [B]*Cancer Research Institute,* [1]*Seoul National University, Seoul, Korea, Republic of,* [2]*Dept of Ob/GYN & Reproductive Biology, Michigan State University, Grand Rapids, MI, United States*

This study was conducted to evaluate the effects of subculture times on cellular competence and cryopreservation capacity of embryonic stem cells (ESCs). Mouse ESCs subcultured either 4 (early passaged) or 19 times (late passaged) were further subpassaged with or without cryopreservation. Cell proliferation, generation of reactive oxygen species (ROS) and incidence of apoptosis were monitored after 1, 2 and 6 subpassages of post-treatment subculture. Faster cell proliferation appeared as shortening of doubling times ($p<0.05$ by PROC-GLM) were detected in the late-passaged ESCs as compared to the early-passaged ESCs, which was accompanied by decreased ATP synthesis. These effects appeared, regardless of cryopreservation, but the difference of each parameter among the treatments disappeared as the post-treatment subculture progressed. The same pattern was detected in ROS generation, but a significant decrease in the generation was observed only in the late ESCs after cryopreservation. There was no remarkable increase in apoptotic cell number between the early-passaged and late-passaged ESCs, but the subculture after cryopreservation further reduced the apoptotic cell number. In conclusion, subculture of ESCs influences cellular activity and subculture repeated at least twice after the designated treatment contributes to the stabilization of ESC activity.

W-2014

A NOVEL POTENTIAL THERAPY FOR VASCULAR DISEASES: DENDRITIC CELLS DIRECT PRO-ANGIOGENIC STIMULATION OF BLOOD-DERIVED STEM/PROGENITOR CELLS

Yael Porat[1], Mark J. Niven[2], Shlomo Bulvik[3]

[1]Hematology BC Stem Cell Research, BioGenCell Sanz Medical Center Laniado Hospital, Netanya, Israel, [A]Bildirici Diabetes Center, [B]Hematology BC Stem Cell Research, [2]Sanz Medical Center Laniado Hospital, Netanya, Israel

Background: Recent data show that dendritic cells (DCs) are important component of stem cell niches in the bone marrow and spleen, and as such may have a role in stem/progenitor cell homeostasis and in pro- and anti-angiogenic processes (Gabrilovich, 1996; Dikov, 2005; Sozzani, 2007). We describe a novel technology for generating a therapeutic population (BGC101) of enriched endothelial progenitor cells (EPCs), using DCs to specifically direct Stem/progenitor cell (SPCs) activity in vitro. This newly developed process makes it possible to use unmobilized blood cells as a source for sufficient numbers of potentially therapeutic stem/progenitor cells within culture for one day.

Methods and results: Selected immature plasmacytoid and myeloid DCs from healthy and diabetic donors, obtained from either the Israel Blood Bank, or Laniado Hospital after receiving signed informed consent approved by hospital's IRB (Bulvik 15/150109), were activated with anti-inflammatory and pro-angiogenic molecules to induce specific activation signals. Co-culturing of activated DCs with SPCs for 12-66 hours generated 83.7±7.4×10^6 BGC101 cells with 97% viability from 250 ml of blood. BGC101, comprising 52.4±2.5% EPCs (expressing Ulex-lectin, AcLDL uptake, Tie2, vascular endothelial growth factor (VEGF) receptor 1 and 2, and CD31), 16.1±1.9% SPCs (expressing CD34 and CD184), and residual B and T helper cells, demonstrated angiogenic and stemness potential and secretion of IL-8, IL-10, VEGF, and osteopontin. When administered intramuscularly to nude mice with limb ischemia, BGC101 yielded a high safety profile and significantly increased blood perfusion, capillary density, and limb function. In the experiment the %perfusion was tested in the vehicle-treated control group, the peripheral blood mononuclear cell (PBMC)-treated group, and the BGC101-1 (1-day culture), BCGC101-3 (3-day culture, high dose), and BGC101-31 (3-day culture, low dose). After 21 days BGC101 treatment doubled the blood flow to the legs from an average of 23±5% after injury to an average of 51±3.1% on day 21 after treatment ($p<0.002$). Cell tracking and biodistribution showed that engraftment was restricted to the ischemic limb.

Conclusions: These observations indicate that alternatively-activated DCs can promote the generation of EPC-enriched SPCs within culture for one day. It is particularly noteworthy that the blood of diabetic patients yielded the same range of numbers and specific cells as that of healthy donors. This is an important finding, as a blood volume of 250 ml can be safely and easily acquired even from patients with anemia. Thus, in addition to its scientific merit the novel technology described here can be expected to facilitate the development of a standardized product with potential clinical applications for treatment of various vascular conditions such as coronary heart disease, stroke and peripheral ischemia.

W-2016

SMAD3 DEPLETED ES CELL TERATOMA AS A NOVEL MODEL TO STUDY TUMOR DEVELOPMENT

Ping Yuan
Chemical Pathology, Li Ka Shing Institute of Health Sciences, the Chinese Univ of Hong Kong, Hong Kong, Hong Kong

Smad3 depleted ES cell teratoma as a novel model to study tumor development

Disease associated gene knockout embryonic stem cells can serve as valuable in vitro models for the study of disease mechanisms and drug screen. Smad3 mediated TGF-β/Activin/Nodal signaling plays important roles in many biological processes and Smad3-/- mice develop colorectal cancer. But, Smad3 depleted embryonic stem cells have yet to be derived. To exam whether Smad3-/- ES cell can be used to study tumor development, we derive Smad3-/- ES cells. Smad3-/- ES cells display no defect on self-renewal and proliferation. They express similar level of pluripotent genes and lineage genes compared to wild type ES cells. Intriguingly, subcutaneous injection of Smad3-/- ES cells into nude mice leads to formation of malignant immature teratomas, while wild type ES cells tend to form mature teratomas. Microarray analysis shows that Rif1, a key DNA repair factor, is highly upregulated in Smad3-/- ES cells. Smad3 binds to Rif1 promoter region and directly represses its expression. As the elevated expression of DNA repair genes is associated with metastasis of tumor, the upregulation of Rif1 may be responsible for the malignancy of Smad3-/- teratoma. This study uncovers a novel mechanism between smad3 and a DNA repair factor Rif1, and also suggests that transgenic ES cell teratoma may serve as a good model to study the mechanism of tumor development.

W-2017

ISOLATION AND CHARACTERIZATION OF ENDOTHELIAL OUTGROWTH FROM CORONARY ARTERIES IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION FOR DISEASE MODELING

Susan Gallogly
Scottish Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, United Kingdom

Background: Endothelial dysfunction is one of the earliest pathological features in atherosclerosis and predisposes to intra-vascular thrombosis, ischemia and infarction. Our understanding of endothelial cell biology is derived mainly from the study of commercially available human umbilical vein endothelial cells (HUVECs). However, HUVECs are not involved in atherosclerosis and provide only limited insight into the pathogenesis of coronary artery disease. We describe a novel method for the isolation of coronary artery endothelial cells from thrombectomy specimens obtained during the treatment of patients with acute myocardial infarction.
Objective: To isolate, expand and characterise coronary endothelial outgrowth (CEO) cells and compare these cells to HUVECs, in order to investigate the potential of CEO cells as a model of human endothelial dysfunction.
Method: Patients presenting to the Royal Infirmary of Edinburgh with ST-segment elevation myocardial infarction (n=15) who underwent emergency percutaneous coronary intervention and thrombus aspiration were recruited. Thrombus specimens were manually dissected, plated onto collagen-I coated plates and maintained in endothelial growth media to encourage cellular outgrowth. Growth kinetics (population doubling time) was evaluated and cells were fixed and immunostained. A multiparameter flow cytometric analysis using monoclonal antibodies to endothelial cell markers was performed (CD31-FITC, KDR-PE, CD146-PE/Cy7 and CD34-APC/Cy; percent positive expression). Angiogenic potential was assessed using an established method of tubule-like structure formation on a basement membrane matrix (Matrigel).
Results: Outgrowth of coronary artery endothelial cells was observed in 9/15 samples. CEO cells were maintained for a minimum of 38 days in culture (mean=4512 days). Populations doubling times of CEO cells were comparable to HUVECs (mean±SD: CEO 2.6±0.5, HUVEC 2.3±0.1 days; p=0.50 student's t-test). CEO cells had typical "cobblestone" morphology and were immunoreactive for the endothelial specific glycoprotein vWF. Surface expression of CD31 and KDR was comparable in both CEO cells and HUVECs (CD31 mean±SD: CEO 74.9±21.3 vs. HUVEC 89.3±11.4, p=0.13; KDR mean±SD: CEO 46.7±31.7 vs. HUVEC 22.2±15.9, p=0.11) whereas CD146 and CD34 expression was increased in CEO cells compared to HUVECs (CD146 mean±SD: CEO 96±5.3 vs. HUVEC 73.5±30.7, $p<0.05$; CD34

mean±SD: CEO 80.9±16.92 vs. HUVEC 40.63±18.06, p<0.001). Limited angiogenic potential was observed in CEO cells with only 4/9 (44%) capable of tubule formation compared to 10/10 (100%) of HUVECs (p=0.01, contingency tables and fishers exact test). CEO cells that were capable of tubule formation had reduced capacity to form connections compared to HUVECs (mean±SD: CEO 12±8, versus HUVEC 63±16 connections; p<0.0001).
Conclusion: Viable coronary arterial endothelial cells can be isolated from thrombus extracted during emergency percutaneous coronary intervention. These cells have a mature endothelial phenotype comparable to control endothelial cells, but have reduced angiogenic potential suggesting they retain the functional characteristics of *in situ* endothelium. This novel approach to isolate dysfunctional endothelial cells may have applications in drug screening and in future studies may provide additional insight into the cellular and molecular basis of endothelial dysfunction in patients with coronary artery disease.

W-2018

IN VIVO AND IN VITRO STUDY OF MIGRATORY CAPABILITY OF HUMAN MESENCHYMAL STROMAL CELLS TOWARDS HEPATOCELLULAR CARCINOMA AND FIBROSIS.

Juan M. Bayo Fina[1], Esteban J. Fiore[1], Flavia Piccioni[1], Mariano Marrodan[1], Marcela Bolontrade[2], Estanislao Peixoto[1], Rodrigo LLoyd[1], Miguel Rizzo[1], Laura Alaniz[1], Jorge Aquino[1], Osvaldo Podhajcer[3], Mariana Garcia[1], Guillermo Mazzolini[1]

[1]Gene Therapy Laboratory, School of Medicine, Austral University, Derqui, Argentina, [2]Laboratory of Molecular and Cellular Therapy, Fundación Instituto Leloir, Buenos Aires, Argentina, [3]Laboratory of Molecular and Cellular Therapy, Fundación Instituo Leloir, Buenos Aires, Argentina

Hepatocellular carcinoma (HCC) represents 85% to 90% of liver primary tumors and is the third-leading cause of cancer-related death in the world. Moreover, the incidence and mortality associated with HCC continues increasing worldwide. In most cases, HCC is developed in patients with liver cirrhosis; for this reason this condition is considered a preneoplasic stage. The hepatocarcinogenesis process involves an extensive exchange of signals between the tumor cells and their microenvironment, which is composed by several cell types, including hepatic stellate cells. Particulary, several proinflammatory cytokines, chemokines and growth factors, which are produced by tumor and stroma cells, are involved in this crosstalk. It is known that mesenchymal stromal cells (MSC) have the ability to migrate in response to most of these soluble factors. The aim of this work was to study the *in vitro* and *in vivo* migration capability of MSCs towards HCC in order to use them for delivery of therapeutic genes. For that purpose, *in vitro* migration was studied by modified Boyden chamber, observing that MSC displayed a higher migration towards conditioned medium (CM) derived from fresh human samples of HCC compared with CM derived from non tumoral tissue. MSCs also showed a high ability to migrate towards CM derived from ex vivo tumors generated in nude mice by the inoculation of HuH7 cell lines or a primary culture of HCC (HC-PT-5). We also found an increased migration to CM derived from liver or tumors derived from animals with liver fibrosis and intrahepatic tumors, when compared with the CM obtained from healthy mice. In order to evaluate hMSC *in vivo* migration, HCC cell line HuH7 was inoculated subcutaneously (s.c.) or intrahepatically (i.h.) in BALB/c nude mice. HuH7 tumors were also developed by intrahepatic inoculation in animals with subjacent fibrosis, induced by the administration of thioacetamide (i.h. TAA). Once tumors have developed, CMDiI-DiR-labeled hMSCs were intravenously injected and biodistribution was monitored by fluorescence imaging (Xenogen In Vivo Imaging System). Seven days later, healthy and tumor bearing mice were sacrificed and MSCs were found in spleen, liver and lungs. Animals with tumors also presented MSCs signal in tumors. Moreover, liver and tumors from i.h. TAA mice showed a stronger signal compared with the other groups. These results were confirmed by microscopy fluorescence visualization of CMDiI+ cells in the isolated organs. Our results indicate that factors produced by HCC and fibrotic liver induce higher hMSC migration both *in vivo* and *in vitro*, compared with lungs and spleen, which showed a lower signal. Taking together, our results indicates that soluble factors present in the tumor milieu induce the recruitment of MSC to HCC, making these cells a promising candidate for effective cellular delivery of therapeutic genes, not only against HCC but also fibrosis.

W-2021

BONE MARROW MESENCHYMAL STROMAL CELLS (BM-MSCs) PROMOTE PANCREATIC CANCER GROWTH AND CLONOGENICITY IN VITRO

Roula Antoon[1], Xing-Hua Wang[2], Yoko Kosaka[3], Sowmya Viswanathan[3], David Hedley[4], Armand Keating[1]
[1]Cell Therapy Program, Princess Margaret Hospital/Ontario Cancer Institute,University Health Network, Institute of Medical Science, University of Toronto, Toronto, ON, Canada, [2]Cell Therapy Program, Princess Margaret Hospital/ Ontario Cancer Institute, University Health Network, Toronto, ON, Canada, [3]Cell Therapy Program, Princess Margaret Hospital/Ontario Cancer Institute,University Health Network, Toronto, ON, Canada, [4]Princess Margaret Hospital/Ontario Cancer Institute,University Health Network, University of Toronto, Toronto, ON, Canada

Introduction: Heterotypic interactions between pancreatic
cancer cells (PCCs) and tumor stroma are critical for pancreatic cancer development and progression. MSCs are multipotent stromal progenitors that may selectively migrate and home to primary and metastatic tumor sites. Little is known about the contribution of BM-MSCs to pancreatic tumor growth and development. Here, we evaluate the paracrine effect of human BM-MSCs on pancreatic tumor growth, apoptosis and clonogenic potential. In addition, we investigate PCC/BM-MSC interactions
via Sonic Hedgehog (Hh) and canonical Wnt signaling mechanisms**.** Hh and Wnt signaling are implicated in pancreatic cancer growth and
progression.
Methods: PCCs from Mia-Paca-2 (undifferentiated), Panc-1 (poorly differentiated) and BXPC3 (moderately differentiated) cell lines were plated on 0.4 um transwell inserts and co-cultured with hBM-MSCs at 1:1 ratio in basic medium with 10% FBS for 48-72 hrs. Tumor cells alone and dermal fibroblast (FB) co-cultures were used as controls. Apoptosis was evaluated by DilC1. Cell proliferation was assessed by Click-iT EdU. Clonogenic assays were performed by culturing 5000 tumor cells in methylcellulose. Experiments were repeated 3 times in triplicate. Hh and Wnt signaling were evaluated by western blot (WB) and RT-PCR.
Results: BM-MSCs significantly promoted the growth of Mia-Paca-2 enhancing tumor cell proliferation by 37% ($P<0.0001$) and reducing
tumor cell apoptosis by 3.4% ($P<0.02$) versus tumor alone but
showed little or no effect on Panc-1 or BXPC3 cell lines. BM-MSCs promoted Mia-Paca-2 cell cycle entry, increasing the number of cells in S phase by 32% ($P<0.005$) compared with tumor cells cultured alone. In addition, BM-MSC co-cultures significantly enhanced the frequency of Mia-Paca -2 colony forming cells in methylcellulose by 1.6 fold versus tumor alone. There was little or no difference between tumor cells cultured alone or co-cultured with FB. The BM-MSC growth promoting effect was associated with increased levels of cyclin E1 and c-Myc proteins in co-cultured tumor cells as shown by WB. RT-PCR and WB show that BM-MSCs have little or no effect on Hh and Wnt activity in co-cultured tumor cells versus controls.
Conclusion: Under our experimental conditions, BM-MSCs promoted pancreatic cancer growth, increased tumor clonogenicity and reduced tumor cell apoptosis in a cell line dependent manner via soluble factors. This growth promoting effect may have been due to increased cyclin E1 and c-Myc expression in Mia-Paca-2 cells in co-culture with BM-MSCs but not via the Hh or Wnt signaling pathways which were similarly active, in Mia-Paca-2 alone, or in co-culture with BM-MSCs. Elucidating the molecular mechanisms by which hBM-MSC support pancreatic tumor growth and the pathways triggered by pancreatic cancer/stromal interactions may lead to identifying new therapeutic targets.

W-2022

MESENCHYMAL STROMAL CELLS (MSCS) IN EXPERIMENTAL SEVERE BURN INJURY: IMPROVEMENT OF SURVIVAL, WOUND HEALING AND MODULATION OF IMMUNE CELLS

CAROLINA C. OLIVEIRA[1], JULIANA U.N. YAOCHITE[1], DANIELA CARLOS[1], REINALDO BATISTA[2], DAIANE F. SANTOS[3], KELEN MALMEGRIM[3], FERNANDO Q. CUNHA[1], MARCO A.C. FRADE[4], EDUARDO A. DONADI[1], JÚLIO C. VOLTARELLI[1]
[A]Biochemical and Immunology, [B]Clinical Analysis and Bromatology, [C]Center of Cell-Based Therapy, [D]Clinical Medicine, [1]Sao Paulo University, Ribeirao Preto, Brazil

Background: Skin damage by severe burns triggers a cascade of events that cause both local effects and systemic responses. These responses lead to a complex biological event involving both resident skin cells and leukocyte subsets. In this context, MSCs are good candidates to cell therapy for burns wounds due to their regenerative potential (differentiation and paracrine effects) and immunomodulatory properties. **Aim:** Evaluating the therapeutic potential of xenogeneic MSCs in the treatment of experimental model of severe burn injury. **Methods:** MSCs were isolated from the bone marrow of FVB GFP+ mice and expanded *in vitro* in α-MEM plus 15% FCS. Wistar rats were submitted to experimental burn through the dorsal contact of a brass bar (200°C for 25s). $5x10^6$ MSCs or PBS were given via intradermal in animals belonging to the treatment group (TG) and the control group (CG), respectively. All analyses were performed at 7, 15, 30 and 45 days after burn. The wound healing area (day 60) was evaluated by the software ImageJ®. $CD4^+$ and $CD8^+$ T cell subsets in the spleen and wound area were evaluated by flow cytometry and neutrophils counts by MPO dosage. Serum levels of IL-10, TGF-β and CINC-1 were examined by ELISA. **Results:** TG showed a higher survivor rate (61.54%) than CG (46.67%), p=0.002. Sixty days after injury the wound healing percentage in TG animals (90.81 ± 5.054; n=7) was higher than in the CG (76.11 ± 3.457; n=7), p = 0.03. MSC treatment induced increased serum levels of TGF-β at day 15 (p=0.02), whereas IL-10 (p=0.05) and CINC-1 (p<0.001) on day 30. We observed an alteration of the CD4/CD8 ratio in CG at 7 (p= 0.004) and 15 days (p=0.035) post-burn, comparing to the TG. We also observed decreased frequency of $CD4^+$ T cells at day 7^{th} (p=0.024), 15^{th} (p=0.031) and 45^{th} post-burned (p=0.008) and decreased frequency of $CD8^+$ T cells at day 7^{th} (p=0.02) post burned (p=0.02), in wound area of TG animals. In this group, we also showed increased neutrophils counts in the damaged area at day 45^{th} after burn (p=0.016). **Conclusion:** These results demonstrate the efficiency of MSCs treatment by improving wound healing and modulating immune cells in severe burns. This evidence may provide the basis for regenerative therapy with allogeneic MSCs as a prompt and effective therapeutic alternative for major burned patients with need of immediate care.

W-2023

ANTIOXIDATIVE EFFECTS OF KINETIN AND EPIGALLOCATECHIN-3-GALLATE IN HUMAN MESENCHYMAL STEM CELLS

Yu-Jen Chang[1], Ji-Ying Leu[2], Mei-Ling Wu[1], Ching-Ping Tseng[2], Shiaw-Min Hwang[1]
[1]Food Industry & Development Inst, Hsinchu, Taiwan, [2]Department of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan

Mesenchymal stem cells (MSCs) are the multipotent stem cells that can give rise to mesenchyme-lineage cells, such as osteoblasts, adipocytes, chondrocytes and myoblasts. MSCs can be isolated from various tissues and have been represented as a promising cell source for cell therapy. It is essential to get the large cell numbers and high activity of MSCs to meet the potential demands for clinical applications. In spite of the strong self-renewal property, MSCs undergo cellular senescence during expanded cultivation in vitro. It is well-known that the oxidative stress induced cell aging during lifespan. In this study, we focused on the relationship between cellular oxidative status and cultivated senescence in bone marrow-derived MSCs (BMMSCs) and cord blood-derived MSCs (CBMSCs). We examined the cellular oxidative stress by culturing cells with antioxidants, kinetin and epigallocatechin-3-gallate (EGCG). To understand the effects of antioxidants supplementation on MSCs, the results of growth status, reactive oxygen species (ROS) value, and lipid peroxidation were analyzed in BMMSCs and CBMSCs. We found that kinetin and EGCG treated MSCs expressed similar surface marker profiles with normal culture condition. The results also showed that the lower oxidative status, the higher catalase activity, and the longer lifespan could be found in kinetin cultivation than control condition in both types of MSCs. However, there were no obvious differences between MSCs cul-

tures with or without EGCG addition. In differentiation test, the osteogenesis could be enhanced by kinetin and the adipogenesis could be inhibited by EGCG in both types of MSCs. Moreover, kinetin but not EGCG decreased the anti-aging marker β-galactosidase in late passage of BMMSCs and CBMSCs. These results indicated that kinetin could be a useful additive to reduce cell oxidative status during MSCs expansion.

W-2024

INTRAVENOUS HUMAN ADIPOSE STEM CELL GRAFTS PROTECT THE BRAIN FROM NEURODEGENERATION, MOTOR AND COGNITIVE IMPAIRMENTS: BIODISTRIBUTION OF HADSC IN YOUNG AND AGED RATS

Sandra A. Acosta[1], Naoki Tajiri[1], MD Shahaduzamman[1], Kazutaka Shinozuka[1], Hiroto Ishikawa[1], Mibel Pabon[1], Lisa Gould[2], Niketa Patel[2], Denise Cooper[2], Yuji Kaneko[1], Paula C. Bickford[3], Cesar V. Borlongan[1]
[1]Neurosurgery, Center of Excellence for Aging and Brain Repair, Department of Neurosurgery and Brain Repair, University of South Florida College of Medicine, Tampa, FL, United States, [2]James A. Haley Veterans Affairs Medical Center, Tampa, FL, United States, [3]Neurosurgery, Center of Excellence for Aging and Brain Repair, Department of Neurosurgery and Brain Repair, University of South Florida College of Medicine. James A. Haley Veterans Affairs Medical Center, Tampa, FL, United States

Traumatic brain injury (TBI) survivors exhibit neurological, motor and neurocognitive symptoms from the primary injury which can become aggravated over time due to secondary cell death. In the present in vivo study, we examined the beneficial effects of human adipose stem cells (hADSCs) in a controlled cortical impact (CCI) model of mild TBI using young and aged (20 months) F344 rats.
Young and aged male F344 rats were treated with 4x106 ADSCs (Tx), conditioned media (CM) or control media (M) at 3 hours post CCI. A separate cohort of animals with the same treatments received DiR-labeled hADSCs. hADSC's labeled with DiR were imaged using the IVIS imager at 1, 4, 12, 24, 48, and 72 hrs post-transplant, with organs separately imaged at the end of the study. At day 0 - 7, post TBI, groups underwent motor tests and at Day 7 cognitive assessment tests, then at day 11, all groups were euthanized and brain tissues harvested.
Significant amelioration of motor and cognitive functions was revealed in young Tx and CM groups but there was less improvement in aged rats Tx relative to TBI rats that received the control media treatment (M). Fluorescent (FL) imaging revealed hADSC's moved to organs and brain within 1 to 12 hours following TBI. In aged rats decreased FL was seen in spleen, however higher FL was observed in brain at 12 - 72 hours post TBI. The impact volume in cortex was found to be significantly reduced in young Tx and CM relative to control treatment. However in aged rats this effect was decreased and only Tx reduced impact volume. Furthermore, the percentage of intact peri-impact area in cortex revealed a significant amelioration in both young Tx and CM treated and old Tx and CM rats in compare with control M. In addition, there was a decrease of hippocampal CA3 pyramidal neuron loss in both Tx young and Tx old rats compared to control M. To further elucidate the mechanism of CM actions, we examined CM from hADSC's grown with antisense RNA to two long noncoding RNA's (lncRNA) known to play roles in gene expression and shown to be secreted by our hADSC's. CM reduced in either NEAT1 or MALAT1 were less effective in preventing motor behavior loss and did not reduce infarct or peri-infarct lesion size.
Results show that hADSCs is a promising therapeutic intervention to rescue against TBI-induced behavioral and histological impairments with better functional recovery in young animals, likely due to robust migration of the transplanted cells to peripheral organs quickly in young animals despite increased stem cell recruitment to the aged ischemic brain. In addition, lncRNA secreted in exosomes from hADSC's are a candidate for mechanism of action of the hADSC's to rescue against TBI-induced injury.

W-2025

STABILIZATION OF BETA-CATENIN IN MTERT-EXPRESSING DENTAL STEM CELLS RESULTS IN SUPERNUMERARY TOOTH FORMATION AND ECTOPIC DENTIN FORMATION

Diana L. Carlone, Rebecca D. Riba-Wolman, Dana M. Ambruzs, Samantha Stewart, David T. Breault
Endocrinology, Boston Children's Hospital, Boston, MA, United States

Adult stem cells play an integral role in the regeneration of a variety of tissues including the mouse incisor. We have recently reported that telomerase (*Tert*) expression marks self-renewing stem cells in blood, intestine and putative bone marrow-mesenchymal stem cells. Based on these findings, as well as a recent report of telomerase activity within putative human dental stem cells, we hypothesized that *mTert* is a biomarker for stem cells within teeth. To investigate whether *mTert*-expressing cells are present in postnatal teeth and function as stem cells in the continual renewal of the mouse incisor, we performed lineage-tracing studies using *mTert*-rtTA :: oTet-Cre :: R26R $^{flox(mT/mG)/+}$ trigenic mice. Our results indicate that *mTert*-expressing cells in the upper incisor give rise to both ameloblasts and odontoblasts indicating that *mTert* expression separately marks epithelial as well as mesenchymal dental stem cell populations. Interestingly, in the lower incisor *mTert* expression marks only mesenchymal stem cells suggesting that discrete stem cell populations exist within each region.
The canonical Wnt/beta-catenin signaling pathway plays an important role in regulating tooth formation and renewal such that activation or repression of this pathway results in altered tooth number and location in both humans and mice. Stabilization of beta-catenin specifically within *mTert*-expressing cells and their progeny using doxycycline-inducible *mTert*-rtTA :: oTet-Cre :: Catnb$^{flox(ex3)/+}$ mice resulted in supernumerary tooth formation at the apical (incisal) end of the incisor. Interestingly, analysis of the incisor dental papilla from these mice showed ectopic dentin formation suggesting that stabilization of beta-catenin within mesenchymal-derived *mTert*+ cells promotes odontoblast differentiation. Finally, preliminary observations indicate these mice also exhibit premature suture fusion and altered calvarial bone formation suggesting *mTert*-expressing cells may play a more global role in craniofacial tissue function/maintenance. Additional studies are currently underway to determine the role of PTEN in regulating Wnt signaling and tooth stem cell function. Supported by the National Institute of Dental and Craniofacial Research (1R21DE022420-01A1) awarded to DLC.

W-2026

VALIDATION OF IN VIVO TUMORIGENICITY TEST FOR THE PROCESS CONTROL OF CELL/TISSUE-ENGINEERED PRODUCTS USING SEVERE IMMUNODEFICIENT NOG MICE

Shinji Kusakawa[1], Kazuhiko Machida[2], Satoshi Yasuda[3], Takuya Kuroda[3], Rumi Sawada[4], Hideki Tsutsumi[2], Shin Kawamata[1], Yoji Sato[3]

[1]Foundation for Biomedical Research and Innovation, Kobe, Japan, [2]Central Institute for Experimental Animals, Kawasaki, Japan, [A]Division of Cellular and Gene Therapy Products, [B]Division of Medical Devices, [3]National Institute of Health Sciences, Tokyo, Japan

Contamination of tumorigenic cells is one of the major concerns in manufacturing process of cell/tissue-engineered products. However, no quantitative evaluation method for tumorigenicity of cell/tissue-engineered products has been established Utilizing of severe immunodeficient mouse strains such as NOD/Shi-scid IL2Rg^{null} (NOG) mice, as xenogenic hosts for tumorigenicity testing to detect a trace amount of tumorigenic cells in products, is worthy of attention. NOG mice have shown highly engraftment potential as compared with conventional immunodeficient mouse strains, such as nude mouse and NOD/scid mouse, which are recommended as rodent xenotransplantation models to assess tumourigenicity of mammalian cells as substrates for the manufacture of biological medicinal products in WHO Technical Report Series, No. 878.. Based on such evaluations, we will be able to assess the capability and the extent of the coverage of the tumorigenicity test. In the present study, to evaluate the sensitivity of the tumorigenicity test using NOG mice, we examined tumor formation of xenotransplanted HeLa cells in NOG (T, B, and NK cell-defective) and the traditionally used nude (T cell-defective) mice. The tumorigenicity of HeLa cells in both immunodeficient mouse strains was evaluated by measuring some parameters that represent the tumorigenic phenotype: tumor-forming capacity, tumor latency and tumor size for 16 weeks. Tumor-forming capacity is defined by quantitative, dose-response, tumorigenicity assays that yield the $\log_{10}$ 50% tumor-producing dose (TPD_{50}) as endpoint values, which represent the limiting or threshold dose of cells that form tumors in 50% of the animals. NOG mice are highly susceptible to tumor induction by HeLa cells (TPD_{50} = 4.1 [approximately 1.3 x 10^4 cells]). In contrast, nude mice showed ~33-fold less tumorigenicity (TPD_{50} = 5.6 [approximately 4.2 x 10^5 cells]) of HeLa cells

compared with NOG mice. NOG mice inoculated with HeLa cells suspended in Matrigel, a basement membrane-like extracellular matrix extract, showed ~5400-fold higher tumorigenicity (TPD_{50} = 1.9 [approximately 80 cells]) of HeLa cells, compared with nude mice. Next, we applied this method to the validation of the sensitivity of tumorigenic cells contaminated in normal human cells (human mesenchymal stem cells (hMSC)). NOG mice were inoculated with HeLa cells (0, 10^1, 10^2, 10^3, 10^4 cells) serially diluted in Matrigel with hMSC (10^6 cells), leading to the TPD_{50} value comparable to that in NOG mice inoculated HeLa cells only. These results indicate that the combination of NOG mice and Matrigel can detect tumorigenic cells with extremely high sensitivity and suggest the usefulness of NOG mice for *in vivo* tumorigenicity test.

Technologies for Stem Cell Research

W-2027

GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM HUMAN CIRCULATING MULTIPOTENT STEM CELLS

Han-Mo Yang, **Ju-Young Kim**, Joo Eun Lee, Hyun-Jae Cho, Yoo-Wook Kwon, Jin Hur, Sang Eun Lee, Sahmin Lee, Jung-Kyu Han, Chang-Hwan Yoon, Young-Bae Park, Hyo-Soo Kim
Seoul Natl Univ Hosp, Division of Cardiology, Seoul, Korea, Republic of

Background: The discovery of induced pluripotent stem (iPS) cells has opened up new possibility of molecular understandings for development and therapeutic applications for patient-specific disease. One of the important issues for clinical applications is cell source. Human peripheral blood is one of the easy accessible cell sources. However, isolated peripheral blood cells have shown low gene transfection efficiency and inconveniences requiring specific methods to isolate. Here, we report a novel population of peripheral blood-derived stem cells, which can be easily reprogrammed to iPS cells.
Methods and Results: We freshly isolated peripheral mononuclear cells (PBMC) from human peripheral blood and seeded on the fibronectin-coated plate. We observed adherent cells from as early as 5 days after the start of culture and those cells gradually formed colonies. We were able to isolate these cells with very high efficiency. Furthermore, we have also confirmed that these cells can be differentiated to osteogenic, adipogenic, and myogenic-lineage cells. Therefore, we named these cells circulating multipotent adult stem cell (CiMS). We were successful in generating iPS cells with these cells. These cells showed enhanced efficiency of gene transduction, compared to the human dermal fibroblast. We obtained reprogrammed colonies (CiMS-iPS) in 8 days after 4 factor virus transduction without feeder cells. We identified CiMS-iPS had similar features to embryonic stem cell in morphology, gene expression, epigenetic state and ability to differentiate into the three germ layers. We obtained more than 40 iPS cell lines from PBMC of patients with cardiovascular disease and normal volunteers.
Conclusions: Our study showed new methods to isolate stem cells from peripheral blood and to generate iPS cells with high efficacy. This suggests that our new approach could be one of ideal methods for clinical application of iPS cells in future.

W-2028

ENGINEERED HUMAN EMBRYONIC STEM CELLS FOR INDUCIBLE GENE EXPRESSION

Vera Alexeeva[1], Wilson Young[2], Carlos Filipe Pereira[1], Ihor R. Lemischka[1], Sunita L. D'Souza[1], **Christoph Schaniel**[3]
[A]Developmental and Regenerative Biology, [B]Medicine, [C]Pharmacology and Systems Therapeutics, [1]Icahn School of Medicine at Mount Sinai, New York, NY, United States

Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) hold great promise for biomedicine. They provide an unlimited source of cells to model not only early human development but also various human diseases. They can be differentiated with varying efficiencies to various mature cell types for the development of disease biomarkers and novel therapeutic strategies. However, the lack of genetic tools similar to those proven useful in dissecting out the complexities of mouse development and differentiation has hampered the realization of the full potential of hESC/hiPSC technology.

In order to develop tools for hESC/iPSC research, we generated hESC lines for inducible transgene expression. We engineered the human H9 ESC line (also known as WA09; NIH registration number 0062) to express M2rtTA constitutively from the human ROSA26 locus on chromosome 3 using transcription activator-like effector nuclease (TALEN) pair to target the human ROSA26 locus between exon 1 and exon 2 with a donor plasmid. This donor plasmid comprised a splice acceptor - M2rtTA - poly adenylation (pA) cassette that is followed by an FRT-flanked phosphoglycerine kinase (PGK) promoter driving hygromycin resistance - pA cassette to facilitate the selection of clones and to allow its subsequent removal in correctly targeted clones. Targeting efficiency using this specific TALEN pair was 86%. Expression and functionality of M2rTA was ensured by infecting the targeted H9 cells with FUWtetO-mOrange lentiviral particles that carry a Doxycycline (Dox)-inducible TetO promoter driving mOrange expression. As expected, all correctly targeted clones showed mOrange expression after Dox-induction (1.5 µg/mL) for 2 days, while mOrange expression was not observed in an incorrectly targeted clone. However, co-infection with FUWtetO-mOrange and FUW-M2rtTA, which confers expression of M2rtTA via the constitutively expressed Ubiquitin C promoter, induced strong mOrange expression after Dox-addition even in this incorrectly targeted line. Ongoing experiments are focusing on investigating the titratability of induction and the ability to induce transgene expression in differentiated cells. In addition, we are targeting the human AAVS1 locus with a cassette that will enable Cre-recombination mediated cassette exchange to place any gene or combination of genes under the control of the Dox-inducible TRE promoter.
This cell line will allow us to study the effect of inducible transgene expression and/or knockdown of various genes during human development and differentiation, thus providing a valuable resource for the stem cell community.

W-2031

AUTOMATED ANALYSIS AND SORTING OF HUMAN iPS CELL CLUSTERS BY LARGE PARTICLE FLOW CYTOMETRY

Francis Smet[1], Paul Wanek[2], Stephanie Sontag[2], Simone Haupt[3], Michael Peitz[3], Rico Bongaarts[1], Oliver Brüstle[3], Rock Pulak[4], **Martin Zenke**[2]
[1]Union Biometrica, Geel, Belgium, [2]Institute for Biomedical Engineering - Cell Biology, RWTH Aachen University, Aachen, Germany, [3]Institute for Reconstructive Neurobiology, Life & Brain, Bonn, Germany, [4]Union Biometrica, Holliston, MA, United States

The generation of induced pluripotent stem cells (iPS cells) from somatic cells is one of the most dynamic fields in biomedicine. iPS cells show appealing promises for disease modeling, pharmacological screening and eventually cell replacement therapy. Yet, to fully take advantage of iPS cell technology several bottlenecks need to be overcome: Traditionally, biotechnology procedures aim at producing one product in large quantities, but iPS cell technology requires the simultaneous production of several hundreds or even thousands of individual iPS cell clones. This is particularly important when it comes to the identification of iPS cell clones, which harbor the disease-specific mutations.
Frequently, standard procedures for iPS cell production are based on manual processing, such as picking of iPS cells and/or clusters. Further approaches use immunomagnetic beads and column-based selection of single cells, which however results in loss of iPS cell clonality. Thus, there is clearly the need to develop procedures and protocols for fully automatic isolation and simultaneous processing of a large number of individual iPS cell clones.
The StemCellFactory project (www.stemcellfactory.de) aims to fully automate by robotics (i) the generation of human iPS cell lines and (ii) their differentiation into cardiomyocytes and neuronal cells. Here we evaluated large particle flow cytometry technology (BioSorter, Union Biometrica) for iPS cell isolation, including multiparametric quality assessment of isolated cells. The large particle flow cytometry instrument is capable of sorting cells and cell clusters ranging from 2-1500 micrometer. The fluid pressures of the instrument (up to 6 psi) are significantly lower than those of traditional flow cytometers, which results in cells/cell clusters being exposed to much lower shearing forces than during conventional single cell flow cytometry.
Human iPS cells were established with Oct4, Sox2, c-Myc and Klf4 reprogramming factors in Sendai virus vectors on feeder layer. iPS cell colonies were stained with the FITC-labeled pluripotency marker TRA-1-60, collagenase treated and cell clusters were subjected to sorting by flow cytometry with the BioSorter device. Cell clusters were sorted

according to size and TRA-1-60 expression and multiple parameters were assessed, and individual cell clusters were deposited in 96 well format. The analysis was fast and dispensing to wells of multiwell plates was fully automated, taking about 20 min for a 96 well plate. Sorted cells were efficiently expanded over several passages as clonal iPS cell lines and evaluated by morphology, expression of pluripotency markers and their growth and differentiation potential. This establishes Biometria's large particle Biosorter flow cytometer as a versatile device for analysis and sorting of primary iPS cell colonies.
The authors for the StemCellFactory Consortium (www.stemcellfactory.de).

W-2032

METALLIC NANOPARTICLES AS LABELS FOR MESENCHYMAL STEM CELLS: EVALUATION OF BIOCOMPATIBILITY.

L. H. A. Silva[1], G. A. Ferreira[2], F. D. Cruvinel[2], E. C. O. Lima[2], R. B. Azevedo[1], J. R. Silva[1], D. M. Oliveira[1]
[1]University of Brasília, Brasília, Brazil, [2]Federal University of Goiânia, Goiânia, Brazil

Recently, labeling and subsequent tracking of stem cells in vivo has risen as a non-invasive alternative technique that answers the best site of inoculation of the cells for a given condition, their pattern of distribution within the organism and how effectively they migrate towards pathologically affected sites. Some examples of materials commonly used as markers are metallic nanoparticles, such as gold and iron oxide nanoparticles. Considering that metallic nanoparticles can be toxic or compromise the physiology / metabolism of receptor cells, it becomes important to conduct preliminary studies of biocompatibility before marking and tracking stem cells in vivo. Thus, our study aimed to test the biocompatibility between gold and iron oxide nanoparticles and human mesenchymal stem cells (hMSCs), by evaluating the amount of cells that remain viable after exposure to different concentrations of the markers: iron oxide nanoparticles were diluted in DMEM low glucose (supplemented with 10% of fetal bovine serum) at 0,015; 0,03; 0,06 and 0,08 mg/mL, whereas gold nanoparticles were diluted at 0,001; 0,0025 and 0,008 mg/ml in the same medium. The hMSCs were incubated with those solutions during 02, 06 and 24 hours. Then, firstly, the MTT (3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyl tetrazolium bromide) cleavage assay was performed to verify changes in the mitochondrial activity of cells. Secondly, to draw a parallel between cellular membrane integrity and the amount of tracer incorporated, "Trypan blue method" and "Prussian blue method" were performed. Ultimately, was made a light microscopy analysis of morphology of hMSCs exposed to the nanoparticles, after 02 and 24 hours, in order to verify signs of apoptosis. The association of the gold nanoparticles with the hMSCs was verified by confocal microscopy while iron oxide nanoparticles, by Prussian blue staining. Our MTT results showed that at least 96% of the cells remained viable even on the higher concentrations of iron oxide tested; whereas the gold nanoparticles also were not toxic: more than 94% of hMSCs remained viable. The same were observed in Trypan blue results: 91% of hMSCs exposed to Iron Oxide remained alive while 96% of cells exposed to gold nanoparticles survived. The morphology of hMSCs remained unchanged regardless of the concentration of nanoparticles and the exposure time, furthermore typical features of apoptosis were not observed. Further studies about the effects of these metallic nanoparticles between on MSCs will be performed, such as electron microscopy analysis, cell differentiation tests and cell proliferation assays, in order to characterize those materials for their use as label to track MSCs.

W-2033

USING ISOGENIC PLURIPOTENT STEM CELL DERIVED MOTOR NEURONS AS A MODEL FOR AMYOTROPHIC LATERAL SCLEROSIS.

Jackson Sandoe, Luis Williams
Harvard University, Cambridge, MA, United States

Amyotrophic lateral sclerosis (ALS) is the most common late-onset motor neuron disease and is characterized by the selective degeneration of cortical and spinal motor neurons. A majority of ALS cases are idiopathic however 10% are familial in nature. Of the familial cases, approximately 20% are due to dominant mutations in SUPER OXIDE DISMUTASE 1 (SOD1). We set out to use stem cell technology as a tool for modeling familial SOD1 ALS in vitro.

Because of documented variability amongst pluripotent cell lines, we used nuclease mediated gene targeting to either introduce an SOD1 A4V-encoding mutation in control human embryonic stem (hES) cells or correct the same endogenous mutation in ALS patient induced pluripotent stem (iPS) cells. We show correct expression of the SOD1 locus in both pluripotent stem cells and motor neurons after gene targeting. A prominent feature of familial SOD1 ALS is the formation of detergent-insoluble SOD1 aggregates. Using motor neurons derived from the isogenic pluripotent cell lines we show the presence of insoluble SOD1 segregating solely with the SOD1 A4V-encoding mutation under conditions of proteasome inhibition. We further investigate other common pathologies of ALS including motor neuron survival and neurite morphology. Our results demonstrate that isogenic pluripotent cell lines provide a valuable resource for ALS disease modeling.

W-2034

LAMININ-521 IS THE OPTIMAL CHOICE FOR PLURIPOTENT CELL CULTURE SINCE IT ENABLES EASY AND RELIABLE SINGLE-CELL PASSAGING OF HES AND IPS CELLS WITHOUT ARTIFICIAL INHIBITORS

Jesper Ericsson, Yi Sun, Kristian Tryggvason
BioLamina, Sundbyberg, Sweden

Laminins are a group of 16 protein isoforms found in the basement membrane in the extracellular matrix. The natural environment for all stationary cells in the body consists of other similar cells and the basement membrane. Laminins are the only tissue-specific proteins in the basement membrane and therefore one critical factor that differentiates one niche from another.
Laminin-521 is one of the first extracellular matrix proteins already expressed by the cells of the inner cell mass in the blastocyst. Our data shows that when using Laminin-521 to create a niche on cell culture plates, pluripotent hES and iPS cells can be grown indefinitely in a pluripotent state. This is the first xeno-free, defined and biologically relevant matrix that truly supports hES or iPS cells in a robust way in cell culture.
Laminin-521 also has growth factor like properties and human ES and iPS cells cultured on Laminin-521 grow twice as fast compared to all other tested matrices. Also due to the biological properties of Laminin-521, stem cells cultured on Laminin-521 can be split 1:20 or up to 1:30 as single cells without the addition of artificial ROCK inhibitor, which can push genetical variations to cell populations.
In conclusion, we show that Laminin-521 is an optimal matrix for hES and iPS cell culture due its biological relevance that allows long-term pluripotent cell growth with single cell passaging without any artificial inhibitors that may modify the cell population.

W-2035

NEW STRATEGY FOR TRACING IMPLANTED STEM CELLS IN VIVO

Jongkai Hsiao[1], Hon-Man Liu[2], Ming-Jium Shieh[3], Min-Feng Wei[4], Chen-Wen Lu[5], Dong-Ming Huang[6]
[1]Department of Medical Imaging, Tzu-Chi General Hospital, Taipei Branch, New Taipei City, Taiwan, [2]Department of Medical Imaging, National Taiwan University Hospital, Taipei, Taiwan, [3]Department of Internal Medical, National Taiwan University Hospital and College of Medicine, Taipei, Taiwan, [4]Institute of Biomedical Engineering, National Taiwan University, Taipei, Taiwan, [5]Department of Life Science, National Taiwan Normal University, Taipei, Taiwan, [6]Center for Nanomedicine Research, National Health Research Institutes, Miaoli, Taiwan

Stem cells gives potential opportunity for tissue repair and organ transplantation. For tissue repair, the cells are delivered into the body either locally or systemically. Visualizing these cells in vivo is thus critical for monitoring its existence and function.
Iron oxide nanopartitles have been used widely for tracing stem cells under magnetic resonance imaging (MRI). Stem cells must ingest plenty amount of nanoparticles for being visualized in MRI. Some critics upon alteration of cell function and behavior has been published. The real existence of the cells has also been challenged since the

cell releases its nanoparticles once if the cell dies. We developed another strategy for monitoring the live stem cells with genetic engineering technique to let these cells shine in MRI.
The following studies are done in vitro and has been proved by the biosafety committee. We use human mesenchymal stem cells from bone marrow and transfect these cells with one novel gene that can capture gadolinium based MR contrast agent. The transfection is inert to cell behavior. The differentiation function is preserved after transfected cell exposed to MR contrast agent. We can observe bright signal that originates from the uptake of gadolinium chelates into the cells. These cells has the ability to transport these gadolinium chelates into extracellular space.
We conclude that transfection of gene that regulates gadolinium chelates is an efficient and practical tool that can be used for cell trafficking. Future animal study is essential for comparison with nanoparticle based trafficking methods.

W-2037

DEVELOPMENT OF HIGH QUALITY cGMP-GRADE HUMAN STEM CELLS FOR HUMAN DISEASE AND TRANSLATIONAL RESEARCH

Shayne Boucher[1], Alex Kharazi[2], Nikolai Tankovich[2], Soojung Shin[1], David Kuninger[1], Mohan Vemuri[1]
[1]Primary & Stem Cell Systems, Life Technologies, Frederick, MD, United States, [2]Stemedica Cell Technologies, San Diego, CA, United States

Quality and functionality of adult stem cells used in human disease research vary considerably due to non-standardized cell derivation protocols. Considerable variance exists with donor source, tissue processing, primary cultivation, and potency, and can generate results that are difficult to replicate and lead to inconclusive findings. To address this issue, we tested various approaches to optimize production of consistent lots of highly purified, rapidly expanding primitive adult stem cells under cGMP conditions that can be utilized as a cell standard or in disease research.
As hypoxia appeared to be beneficial for hMSC cultures (Ma et al 2009; Das et al 2010; Basciano et al 2011), we compared hMSC expanded under normoxic (20% O_2) and hypoxic (5% O_2) conditions. At Passage 2 and 4, hMSC yielded 2 fold and 1.5 fold increase in cell number when cultured under hypoxic condition as compared to normoxic condition. We also observed 1.7 fold increase in the number of colonies in CFU-F assays when cultured under hypoxic condition. Further expansion was realized when we incorporated media components with good lot-to-lot consistency and high unit activity.
Hypoxic hMSC should meet minimal criteria for multipotent MSC (Dominici et al 2006). Therefore, we analyzed hypoxic hMSC by flow cytometry for evidence of >95% MSC surface markers (CD73, CD90, CD105, CD166), <5% non-MSC surface markers (CD14, CD19, CD34, CD45), and <5% MHC class II marker (HLA-DR). At all passages, the hMSC exceeded minimal criteria. Differentiation studies confirmed ability of hypoxic hMSC to generate adipogenic, osteogenic and chondrogenic lineage cell types. In addition, a karyotype analysis was performed and no gross abnormalities were observed.
Since there is considerable interest in reparative capacity of hMSC, we performed a cytokine profile on normoxic and hypoxic hMSC. For angiogenic factor VEGF and chemoattractive factor SDF-1, we observed a significant increase under hypoxic condition versus normoxic condition (VEGF: 9.17±0.67 vs 7.67±0.86 ng/millCells/24 hours; SDF-1: 12.2±1.01 vs 9.2±1.27 ng/millCells/24 hours). These increases suggest that hypoxia endows hMSC with angiogenic- and migratory-inducing properties.
By employing an optimized cultivation and potency testing protocol during scale up expansion, we can start with 2 million cells from a Passage 2 master bank and yield 1.5 billion cells at Passage 4. This translates into a working bank size of 100 vials of 1.5e7 viable cells/vial within 14 days. We have established consistency of this system over forty lots of Passage 4 cells.
We have demonstrated the expansion of adult stem cells at larger scale following optimized standardized protocols without losing their intrinsic stem cell nature. With this source of well-characterized, consistent and potent adult stem cells, investigators can make meaningful comparisons in their disease research studies, drug screening assays,

and design of effective cell-based therapies. These cells are now being used or will be used in Phase I/II clinical trials (ClinicalTrial.gov ID NCT01297413, NCT01770613, NCT0177167). Similar scale up approaches are being applied to human neural stem cells and human retinal pigment cells for the research and treatment of neurologic and ophthalmic disorders.

W-2038

ENHANCED HOMING OF MESENCHYMAL STEM CELLS TO BRAIN TUMORS

Christopher Smith[1], Benjamin Lin[1], Saksham Gupta[2], Thomas O'Donnell[2], Sagar Shah[1], Kaisorn Chaichana[3], Alfredo Quiñones-Hinojosa[3], Andre Levchenko[1]

[A]Biomedical Engineering, [B]Neuroscience, [C]Neurosurgery, [1]Johns Hopkins University, Baltimore, MD, United States

Introduction: Brain cancer is a devastating ailment, leading to more than 1,300 deaths in the United States each year. Glioblastoma (GB) is the most common and aggressive type of primary brain cancer in adults. Despite the use of standard treatments, such as radiation, chemotherapy, and surgical resection, GB displays characteristic recurrence, followed by swift fatality within two years. In the search for more effective therapies, recent studies have specifically targeted multipotent adult mesenchymal stem cells (MSCs) to brain tumor (glioma) microenvironments. MSCs have obtained significant interest as drug delivery tools due to their intrinsic tropism for tumors in vivo. The ability of MSCs to breach the blood brain barrier (BBB) provides the unique potential to treat brain disorders, namely brain cancer. Despite these advantages, the efficiency of MSC homing to the brain has been limited, hindering the feasibility of such therapies. We hypothesize that modulation of MSCs can enhance their delivery to the brain.

Methods: Our studies employ commercial and primary-cultured human adipose-derived mesenchymal stem cells (hAMSCs), which benefit from less invasive isolation than bone marrow-derived cells. Utilizing novel micro- and nanotechnology, e.g. microfluidics and patterned cell substrates, we developed intricate in vitro models to analyze the multi-step process by which hAMSCs reach tumors. Our experiments have specifically investigated the putative MSC homing steps of firm adhesion, transendothelial invasion, migration, and chemotaxis. We analyzed effects on these processes by soluble factors in glioma-conditioned medium (GCM), and by the extracellular matrix (ECM) proteins, fibronectin and laminin. Finally, we examined the effects of these factors on homing in vivo using orthotopic animal models of human glioma.

Results: Through exposure of hAMSCs to soluble and immobilized proteins, we have observed enhanced homing in vitro. hAMSC adherence to blood vessel endothelium, measured by numbers of flowing cells that attach to endothelial monolayers, is improved following pre-exposure to GCM and fibronectin ($p \leq 0.05$). After this preconditioning, hAMSCs also demonstrate enhanced transendothelial invasion towards GCM through a model of the BBB ($p \leq 0.05$). Other results show enhanced migration (measured by speed, alignment, and persistence) of hAMSCs pre-exposed to GCM and laminin ($p \leq 0.05$). In addition, hAMSC chemotaxis towards GCM gradients intensifies following GCM preconditioning ($p \leq 0.05$). Combining these treatment techniques in vitro induced enhancements in all of the homing steps described above ($p \leq 0.05$). Preliminary in vivo results suggest increased localization of hAMSCs to glioma xenografts in mice, following combined pretreatment techniques, and subsequent injection into the bloodstream.

Conclusions: Our results demonstrate that specialized culturing methods can enhance specific elements of the MSC homing process, which may lead to in vivo applications. This approach mirrors previous reports that have improved engraftment to other target tissues, e.g. the heart. Uncovering methods to improve tissue-specific MSC localization could revolutionize drug delivery for various diseases.

W-2041

HIGH THROUGHPUT PRODUCTION OF HUMAN MESENCHYMAL STEM CELL SPHEROIDS WITH CONTROLLABLE MICROENVIRONMENT VIA DOUBLE-EMULSION BASED DROPLET MICROFLUIDICS

Hon Fai Chan[1], Ying Zhang[1], Yi-Ping Ho[2], Youngmee Jung[1], Ya-Ling Chiu[1], Kam W. Leong[1]

[1]Biomedical Engineering, Duke University, Durham, NC, United States, [2]Interdisciplinary Nanoscience Center (iNANO),Biomedical Engineering, Aarhus University, Aarhus, Denmark

Multicelluar spheroids play an important role in stem cell research as they better recapitulate three-dimensional tissue than conventional monolayer culture in enabling greater cell-cell and cell-matrix interactions. Moreover, exogenous substrates and growth factors also contribute to directing stem cell behavior. As such, there is an interest for high-throughput generation of stem cell spheroids along with fine tuning of their differentiation potential via controlling exogenous substrate and growth factors supplied, which can be achieved in microfluidics system. This study reports the application of microfluidics-generated double-emulsion (DE) droplets, with a configuration of water-in-oil-in-water, as a picoliter-sized bioreactor for rapid cell aggregation and control of microenvironment for spheroids culture. Human mesenchymal stem cells (hMSC) assembled to form single aggregate in the droplets within two hours and could be subsequently released with over 95% viability. The size of hMSC spheroids could be controlled by varying the input cell density. Precursor gel solution can be adopted as the inner phase to produce spheroid-encapsulated microgels after spheroid formation. The selectively permeability of the oil layer retains growth factors within the droplets, which can subsequently be encapsulated in the microgels for controlled release. The encapsulation of hMSC spheroids in alginate and alginate-RGD hydrogel has been demonstrated to facilitate hMSC differentiation towards various lineages. In summary, the technology facilitates high-throughput production (20Hz) of hMSC spheroids with minimal reagent input including gel solution and growth factors in picoliter-sized DE droplets for fine tuning of microenvironment. The compatibility of this technology with different hydrogels system renders it attractive for screening optimal microenvironmental conditions to advance stem cell tissue engineering.

W-2042

A SERUM-FREE, XENO-FREE, DEFINED MEDIUM FOR EFFICIENT EXPANSION OF HMSCS ON A SYNTHETIC SURFACE

Paula Dolley-Sonneville, Lori Romeo, Zara Melkoumian
Corning Incorporated, Corning, NY, United States

There is a great interest in application of human mesenchymal stem cells (hMSCs) in cell therapy and tissue engineering due to their properties of self-renewal, multipotency, immunomodulation, and trophic potential. One of the challenges faced in the clinical application of hMSCs is the need for efficient *in vitro* expansion of these cells without altering their properties.

Traditionally, hMSCs are cultured *in vitro* on tissue culture treated (TCT) plastic in culture medium supplemented with fetal bovine serum (FBS). Due to the undefined nature of FBS, there is variability in hMSC cultures, as well as risk for pathogen contamination, a major safety concern for clinical applications.

To address these concerns, several chemically-defined, serum-free media have been developed and commercialized. However, most of these media require pre-coating of culture vessels with biological substrates, such as fibronectin, to enable hMSC adhesion in serum-free conditions. Coating of culture vessels with biological substrates is challenging and labor intensive for large scale cell production and can also contribute to variability in cell performance.

This study describes a novel serum-free, xeno-free, defined medium, Corning® stemgro® hMSC medium, for hMSC culture. Stemgro hMSC medium enables efficient attachment and expansion of hMSCs without the need for biological coating of culture vessels. Cell performance (cell number, viability, population doublings, immunophenotype, and multipotency) in stemgro hMSC medium was compared to cells cultured in traditional 10% FBS medium. Our results show significantly higher yield (>100 fold) of human bone marrow derived MSCs (hBMSCs) when cultured in stemgro hMSC medium compared to 10% FBS. Importantly, hBMSC expanded in stemgro hMSC medium for >5 serial passages retained stable doubling time, typical elongated spindle-like morphology, surface markers profile, normal karyotype, and multipotency.

We believe stemgro hMSC medium in combination with the synthetic surface (Corning® CellBIND® Surface) provides a complete serum-free, xeno-free, defined culture environment for efficient expansion of high quality hMSCs for both research and therapeutic applications.

W-2043

EXPANSION OF ADULT BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IN THE QUANTUM SYSTEM PRODUCES THERAPEUTIC DOSES UNDER HYPOXIC CONDITIONS

Boah Vang, Brian Nankervis, Kim Nguyen
Terumo BCT, Lakewood, CO, United States

The large numbers of *ex vivo* expanded cells that are required in many clinical cell therapy protocols (>100 million per patient) make standard culture conditions problematic and expensive, resulting from the need for extensive personnel and facility resources and the high potential for contamination. To meet such clinical demand, a robust, automated and closed cell expansion method is optimal. The Quantum Cell Expansion System (Quantum System) is a functionally closed, automated hollow fiber bioreactor system designed to reproducibly grow adherent cells in either Good Manufacturing Practice (GMP) or research laboratory environments. The Quantum System has successfully been used for the *ex vivo* expansion of clinical-scale quantities of adult bone marrow-derived mesenchymal stem cells (MSC) under normoxic conditions.
This study was a proof-of-concept that MSC may be ex cultured on the Quantum System using different coating reagents [fibronectin (FN, BD Biosciences) and cryoprecipitate (CPPT, Bonfils)] in a hypoxic environment of 5% O_2, 20% CO_2, and balance N_2. Three different donors were expanded for multiple passages. Bioreactor coating with FN or CPPT, cell loading, attachment, feeding, and harvest followed standard Terumo BCT protocols developed for the culture of MSC. Gas concentrations in the closed-loop perfusion system was carefully monitored and characterized. Experimental results demonstrate that therapeutic doses of MSC can be achieved under hypoxic conditions in the Quantum System while maintaining phenotypic characteristics of MSC by flow cytometry, morphology, and tri-lineage differentiation as defined by ISCT.

W-2044

GENERATING OPTIMAL PSEUDOURIDINE AND 5-METHYLCYTIDINE MODIFIED MESSENGER RNAS FOR IPSC REPROGRAMMING

Jiehua Zhou[1], Maggie Bobbin[1], Julie Escamilla-Powers[2], Eric Arnold[3], Anthony Lauer[4], Laura Juckem[4], Karl-Dimiter Bissig[5], **Anton McCaffrey**[2], John Rossi[1]
[1]Department of Molecular and Cellular Biology, Beckman Research Institute of the City of Hope, Duarte, CA, United States, [A]Research & Development, Cell Biology, [B]Chemistry, [2]TriLink BioTechnologies, Inc., San Diego, CA, United States, [3]Mirus Bio, Madison, WI, United States, [4]Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX, United States

Recently, there has been significant interest in messenger RNA (mRNA) for gene therapy applications as well as for the generation and manipulation of stem cells. Several groups have shown that mRNAs are attractive vehicles for therapeutic gene expression in mammals. Additionally, Warren et al. demonstrated highly efficient induced pluripotent stem cell (iPSCs) generation by transfection of mRNAs encoding reprogramming factors. mRNAs have no risk of insertional mutagenesis and subsequent oncogenesis. Thus, the authors suggested that iPSCs generated using mRNA should be safer than iPSCs derived by plasmid transfection or viral transduction.
A key insight regarding mRNA expression systems was the recognition that mRNAs induce innate immune responses in transfected cells. Kariko et al. showed that substitution of uridine and cytidine residues with pseudouridine and 5-methlycytidine dramatically reduced innate immune recognition of mRNA and pseudouridine modified RNA was translated more efficiently and had increased nuclease resistance.
These studies highlight the importance of development of stable, non immunogenic mRNA to support these applications. In this current study, we synthesized capped pseudouridine and 5-methlycytidine modified mRNAs expressing Klf4, c-Myc, Sox2, Lin28, Oct4, Luciferase and GFP at 10 mg scales. Fluorescence activated cell sorting (FACS) demonstrated >95% transfection in HEK-293 cells. Expression in BJ fibroblasts, MRC-5 fibroblasts, human CEM T-cells and CD34+ hematopoietic stem cells was also achieved. Transfected mRNAs showed a surprising long duration

of expression (up to 8 days post-transfection). We compare the transfection efficiency and toxicity associated with 14 days of repeated transfection of fibroblasts using several transfection reagents and find high transfection efficiency and low toxicity with TransIT®-mRNA (Mirus Bio). Repeated transfection with Klf4, c-Myc, Sox2, Lin28 and Oct4 produced robust stem cell colonies. Similar methodologies are applicable to the generation of differentiation factor mRNAs to drive iPSCs down different lineages.
Gene therapy applications of mRNA will require scalable purification methods that are able to produce mRNAs at gram scales. Recently, it was shown that purification of mRNA by HPLC dramatically reduced innate immune responses relative to unpurified mRNA. Here we compare mRNA purified by classical silica membrane chromatography to HPLC purified materials. HPLC reveals that in vitro transcribed mRNAs may contain complex mixtures of subspecies.

W-2045

AN OXYGEN GRADIENT LANDSCAPE GENERATED IN A MICROFLUIDIC DEVICE DEMONSTRATES CROSSTALK BETWEEN NORMOXIC AND HYPOXIC STEM CELLS

Megan Rexius[1], Asrar B. Malik[2], Peter Toth[2], Navdeep S. Chandel[3], David T. Eddington[1], Jalees Rehman[2]
[A]Bioengineering, [B]Pharmacology, [1]University of Illinois at Chicago, Chicago, IL, United States, [2]Medicine, Northwestern University, Chicago, IL, United States

Tissue ischemia is characterized by gradients in the level of oxygen ranging from extreme hypoxia at the primary site of injury to reduced ischemia and even normoxia at the edges or penumbrae. Tissue hypoxia is the primary physiological stimulus for angiogenesis. Homogenous hypoxia is known to increase secretion of pro-angiogenic vascular endothelial growth factor (VEGF) expression in human mesenchymal stem cells (MSCs) which can activate angiogenesis in endothelial cells. Here using a novel microfluidic device in which oxygen gradients could be finely tuned to simulate varying levels of tissue ischemia, we addressed the question whether paracrine crosstalk occurs between cells exposed to varying oxygen levels by studying close proximity interactions between normoxic and hypoxic cells.
Our cell culture platform with precise control of oxygen gradients allows different oxygen levels to be selectively applied to cell types in a co-culture (e.g. one cell type can be hypoxic while the other is normoxic), while permitting paracrine interactions between the distinct cell types via shared cell culture media. The gas-permeable cell culture platform was designed as an integrated, multilayered device. Validation of the oxygen profile of the gradient was performed using a fluorescent oxygen sensor. Production of pro-angiogenic vascular endothelial growth factor (VEGF) was used as a readout to determine whether normoxic cells influence nearby hypoxic cells. Immunofluorescent staining and quantitative PCR were used to assess alterations in gene expression in an oxygen gradient versus homogenous oxygen levels. An oxygen-dependent-domain green fluorescent protein construct enabled real-time monitoring of Hypoxia Inducible Factor (HIF) expression.
We observed that hypoxia-induced upregulation of VEGF in MSCs was suppressed by close-proximity normoxic MSCs. Also in co-cultures of hypoxic MSCs and normoxic microvascular endothelial cells (MVECs), normoxic endothelial cells prevented VEGF expression. Importantly, hypoxic upregulation of the glucose transporter (Glut1) expression, a HIF-induced gene, in MSCs was not affected by the normoxic cells. The abrogation of the response in the hypoxic cells was, therefore, not the result of inhibition of a HIF response. Thus, neighboring normoxic cells suppress cellular hypoxia-induced VEGF expression in MSCs via the release of transmissible factors. These findings suggest the ability of MSCs in ischemic tissue to promote endothelial cell proliferation and tissue regeneration through VEGF generation is dictated by the release of paracrine factors by the nearby normoxic MSCs. In this sense, the oxygen landscape into which MSCs are transplanted is likely to be a key determinant of their regenerative function. Our microfluidic cell culture platform can be leveraged to study paracrine interactions across an oxygen gradient. Our ongoing experiments are now applying this technology to study hypoxic responses of embryonic and induced pluripotent stem cells in an oxygen landscape.

W-2046

MICRORNA PROFILING AS A QUALITY SIGNATURE FOR CELLULAR THERAPIES

Vincent O'Brien, Daria Olijnyk, David Mallinson, Sarah Paterson, Susan Ridha
Sistemic Ltd, Glasgow, United Kingdom

Stem cells derived from embryonic or adult tissue and from reprogrammed somatic (iPS) cells have significant promise for regenerative medicine. However, despite similarities in developmental potential, several groups have found fundamental differences between stem cell lines that could impact on the potency and/or safety of the resultant cell populations but which were not predicted using current monitoring procedures based on flow cytometry and analysis of panels of mRNAs. There is a requirement for reliable tools to monitor cell populations during the processes of stem cell line development, directed differentiation and scale-up to safe, therapeutically-useful cell populations. MicroRNA (miRNA) profiling is proving highly informative for cell characterisation and cell therapy developers are now exploring using miRNA profiling for product characterisation. Using a variety of miRNAome discovery technology platforms combined with subsequent verification and validation using alternative technologies, Sistemic has developed a novel, reliable, generic monitoring tool (SistemQC™) that provides both a robust indication of cell identity, homogeneity and potency as well as providing insights into the underlying changes in gene expression associated with observed biological phenotypes. SistemQC™, utilises a combination of microRNA expression profiling and customised, multi-layered data analysis to provide a simple, robust and cost-effective tool to monitor the maintenance of pluripotency in stem cell lines across passage, the staging of directed differentiation from embryonic, iPS or direct reprogramming strategies and, post scale-up, an assessment of functional attributes and safety profile of the cells. Data will be presented to support these applications.

W-2047

MEDIUM-THROUGHPUT MICRORNA SCREENS FOR THE IDENTIFICATION OF MOLECULAR TARGETS AND MECHANISMS LINKED TO HUMAN PLACENTA-DERIVED ADHERENT CELL HEALTH

Kathy Karasiewicz-Mendez[1], Xu Dong Lee[2], Timothy Maguire[2], Martin Yarmush[2], Robert Hariri[1], Stewart Abbot[1], Xiaokui Zhang[1]
[1]Celgene Cellular Therapeutics, Warren, NJ, United States, [2]Rutgers University, Piscataway, NJ, United States

Background: MicroRNAs (miRs) are small, non-coding RNA molecules that regulate gene expression at the level of translation. The binding of miRs to complementary regions in the 3' untranslated regions of their target mRNAs results in translational interference or transcript degradation. A striking feature of miRs is the ability of single miRs to target multiple genes and pathways, with the potential to affect numerous biological functions or responses. Herein we describe a medium-throughput human miR- mimic library screen (1,090 miRs), and subsequent bioinformatics analysis, to identify the molecular targets and mechanisms linked to Placenta-derived adherent cells (PDAC) responses to cellular stress conditions.
Methods: Cell health was deliberately perturbed by overnight incubation of PDAC with 100% normal rat serum and apoptosis was monitored by measuring caspase 3/7 activity.
For primary screening, PDAC were transfected with the Ambion Pre-miR Mimic Library (miRBase v15) for 24 hours prior to overnight exposure to 100% normal rat serum. PDAC were stained with Hoechst 33342 and imaged using an InCell Analyzer 2000 (GE) to enumerate nuclei and cells, followed by staining with Caspase-Glo 3/7 to assess caspase 3/7 activity/cell. Hits were defined as miRs that significantly lowered PDAC caspase 3/7 activity/cell vs. either of the Ambion negative controls ($p<0.05$, Student's t-Test).
Secondary confirmation studies included repetition of caspase 3/7 activity/cell assays in combination with additional assessments of metabolic activity and mitochondrial membrane potential. Bioinformatic analyses were used to generate a list of putative target genes of the confirmed miR hits, prior to further validation using Taqman Gene Expression Assays, siRNA knock-down, cell cycle analysis, and flow cytometric measurements of cell cycle proteins.
Results and Conclusions: Our results demonstrated that exposure of PDAC to 100% rat serum significantly increases caspase 3/7 activity, decreases metabolic activity, and decreases mitochondrial activity. We have identified three miRs (miR-29a, miR-16, and miR-424) that alleviated cell stress as indicated by a significant decrease in Cas-

pase 3/7 activity. Secondary screening confirmed that modulation of caspase 3/7 was associated with increased metabolic activity and increased mitochondrial membrane potential. Target gene prediction for these miRs identified hundreds of genes for subsequent pathway analyses and has indicated that down-regulation of cyclins and other cell cycle regulation genes, and induction of quiescence, could protect against specific inducers of cell stress e.g. exposure to rat serum. Furthermore, gene expression studies together with cell cycle and cyclin analysis of PDAC transfected with miR mimics of miR-29a, miR-16, and miR-424 support this hypothesis. Modulation of miRs and their targeted genes/pathways may represent novel approaches for maintaining or augmenting cell health.

W-2048

MOLECULAR IMAGING OF THE MESENCHYMAL STEM CELLS MIGRATORY PROPERTIES TOWARDS DIFFERENT PATHOLOGIES.

Carolina Belmar-Lopez[1], Georges Vassaux[2], Jerome Burnet[3], Juan Carlos Ramirez[4], Miguel Quintanilla[5], Antonio de la Vieja[6], Santiago Ramon y Cajal[7], Javier Hernandez-Losa[8], **Pilar Martin-Duque**[9]

[1]I+CS. Instituto de Investigación Sanitaria de Aragón, Zaragoza, Spain, [2]Université de Nice Sophia Antipolis, Nice, France, [3]Cancer Research-UK, London, United Kingdom, [4]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain, [5]Instituto de Investigaciones Biomédicas "Alberto Sols" CSIC, Madrid, Spain, [6]ISCIII, Madrid, Spain, [7]Pathology, Hospital Vall d'Hebron, Barcelona, Spain, [8]Pathology, Hospital Vall d´Hebron, Barcelona, Spain, [9]Biotechnology, Francisco de Vitoria´s University/ I+CS. Instituto de Investigación Sanitaria de Aragón/ Fundación Araid, Madrid, Spain

Bone marrow-derived mesenchymal stem cells (MSCs) are multipotent adult stem cells from mesodermal origin that are present within the bone marrow stroma. MSCs are capable of multilineage differentiation under appropriate stimuli. Due to their ease isolation and their pluripotent nature, MSCs are an ideal source for clinical and regenerative medicine approaches in a great variety of applications. One characteristic of MSCs is their capacity to migrate and to proliferate within areas of inflammation and tumours, as part of the tissue remodelling process.
STUDY´S OBJECTIVE: In this report, we demonstrate that when labelled mesenchymal stem cells are injected systemically by intravenous injection and simultaneously into different animals with various pathologies, they tend to migrate naturally to the damaged tissue, independently of the pathology.
METHODS: To prove this hypothesis, we performed in vitro and in vivo imaging techniques by using a broad range of reporter genes (GFP, hNIS and R-Luc) and non-invasive techniques (PET, BLI or fluorescence).
RESULTS: MSCs migrated to the expected target organs and engrafted tumours, targeted the pancreas (in case of diabetes) or went to the skin (in case of injury/wound healing). We were not able to detect signalling in any other organs at the different time points, but changes in the intensity of the signal along the course of the experiment were observed.
CONCLUSIONS: The results suggest that MSCs possess migratory capacity to damaged areas independently of the pathology and that they could be used to enhance tissue repair.

W-2051

QUALITY CONTROL AND TRACEABILITY OF PLURIPOTENT STEM CELL CULTURE THROUGH AUTOMATED COLONY IMAGING

Audrey Nifenecker[1], Olivier Feraud[2], Eva Planes[1], Nicolas Bensid[1], Elsa Raban[1], Emilie Gobbo[2], Noufissa Oudrhiri[2], Annelise Bennaceur-Griscelli[2], **Yates Frank**[1]

[1]CellTechs Research Laboratory, Sup'Biotech School for Bio-Engineering, VILLEJUIF, France, [2]ESTeam Paris Sud Core Facility, INSERM UMR_S935, Univ Paris Sud 11, VILLEJUIF, France

Because pluripotent stem cells can self-renew and proliferate indefinitely, it is essential to ensure that regular quality control steps are performed to assess their healthy and pluripotent status. Quality characterization of pluripo-

tent stem cells usually relies on a standard series genetic (karyotyping, CGH⋯), phenotypic (immunostaining, gene expression analysis...) and functional (teratoma, in vitro diffentiation...) assays. However, one key method used in laboratories consists in visually inspection of the morphology of stem cell colonies during cell culture under the microscope. Although not sufficient in itself to provide a functional assessment of the quality of a cell line in culture, the morphology of unstained colonies remains the easiest and most common method of qualifying the status of pluripotent stem cells in culture. Laboratories working with induced pluripotent stem cells (IPSC) end human embryonic stem cells (hESC) are however confronted with the lack of objective, user-independent parameters to describe the morphological criteria associated with undifferentiated, fully pluripotent stem cells.
We present here a visual atlas of IPSC colonies representative of the states which can be observed during long term culture of IPSC, and propose a nomenclature in order to establish a consistent description of the morphological characteristics of each colony. By taking advantage of an automated microscopy technique (CellCelector, ALS) we also propose a strategy to document the status of a cell culture based on whole-plate phase contrast microphotography. Whole plate microphotography can be used to individually trace, identify and grade all the colonies present on a cell culture plate. By attributing a “pluripotent morphology score” to whole cell plates, rather than a randomized manual visual inspection, we thus endeavour to be able detect the atypical derivation of IPSC lines which can occur during culture, mostly due to genetic or epigenetic abnormalities. This work will be the basis for setting up a computer-assisted colony scoring system via Pattern Recognition Image Analysis. Associated with our morphological scoring system, the CellCelector automated system may also be used to perform automated or semi-automated mechanical IPSC colony picking and passaging, thus ensuring a reliable and reproducible method for long-term culture and batch amplification of IPSC.

W-2052

EXTRACTING NORMALIZING GENES IN MOUSE EMBRYONIC STEM CELLS FROM SINGLE CELL RNA SEQUENCING DATA

Matan Sorek, Eran Meshorer
The Hebrew University of Jerusalem, Jerusalem, Israel

Measuring gene expression is a fundamental requirement in biology. Quantifying expression at the transcript level usually involves quantitative reverse-transcription PCR (qPCR). In the stem cell field, experimental variability is particularly high, because it frequently involves differentiation to different cell types, which change their entire expression map. Therefore, finding good controls for standardization and for the elimination of experimental variability is a difficult task. Two different approaches were previously proposed, namely, to normalize to one or more reference genes, and to normalize to the input RNA. Since the latter method is more complex and does not resolve the variability induced by the reverse-transcription process, identifying stable reference genes is superior. Previous studies addressed this by selecting a small set of ‘housekeeping’ genes. These were then ranked, using qPCR, according to their stability, typically based on their pairwise correlations. Recent advances in high-throughput sequencing methods enabled the development of whole-transcriptome single cell RNA-seq approaches, which provide an unprecedented opportunity to develop advanced, unbiased normalizing algorithms. Here we developed an approach that utilizes this information to select normalizing genes more accurately. Analyzing 3 different single cell RNA-seq datasets, we find new reference genes in mouse embryonic stem cells (mESC), which are significantly more reliable than the commonly used reference genes. We demonstrate that commonly used genes such as Gapdh, Actin and Tubulin show high variability when compared with our novel normalizers. We also expand this analysis and provide novel general reference genes for a wider range of cells, not limited to the stem cell field. Our algorithms have thus every potential to set new guidelines for standardization in studies involving gene expression measurements.

W-2053

EFFICIENT DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO PAX7 POSITIVE MUSCLE PROGENITORS USING PROTOCOLS DERIVED BY MULTIPLEXED COMBICULT TECHNOLOGY

Marina Tarunina, Jey Jaykumar, Davide Danovi, Tom Watson, Yen Choo, Massimiliano Cerletti

ProgenitorLabs Ltd & Plasticell Ltd, Stevenage Bioscience Catalyst, Stevenage, United Kingdom

Muscle progenitors derived from human embryonic stem (hES) cells offer a renewable source of cells for drug screening and potentially for cell therapy. Here we describe the development of novel defined feeder-free protocols to direct differentiation of hES cells into skeletal myogenic progenitors with the ability to further differentiate into functional muscle. New differentiation protocols were identified through a proprietary combinatorial cell culture technology (CombiCult®, Plasticell). hES cells were cultured on microcarrier beads through more than 10000 distinct combinations of culture conditions to identify protocols that resulted in high differentiation efficiency into muscle progenitors. Tracking of conditions was achieved via concomitant labeling of microcarrier beads using nanomaterial tags. Isolation of microcarriers bearing Pax7 positive cells and tag deconvolution indicated protocols for mesoderm induction, mesoderm differentiation and myogenic lineage differentiation. Muscle progenitors produced by highly efficient protocols were further characterized with respect to cell surface marker expression and regenerative properties. We describe an innovative stem cell technology that allows exploration of signaling pathway regulation with small molecules during stem cell differentiation. Its use with skeletal muscle in particular will speed up the derivation of cells important both for drug screening and for applications in cell therapy for neuromuscular disorders.

W-2054

NOVEL MARKERS OF HUMAN MIDBRAIN DOPAMINE NEURONS AS IDENTIFIED THROUGH BAC TRANSGENIC MOUSE EMBRYONIC STEM CELLS

Yosif M. Ganat
Developmental biology, MSKCC, New York, NY, United States

In this study we used 3 BAC transgenic mESC reporter lines representing distinct stages of midbrain dopamine (mDA) neuron development - Hes5::GFP (early), Nurr1::GFP (mid), and Pitx3::YFP (late). After two weeks of differentiation, FACS-purified cells were subjected to microarray analysis under two different paradigms: interline (i.e. Pitx3+ vs Hes5+) and intraline (i.e. Nurr1+ vs. Nurr1-). Gene ontology and in vivo experimentation lead to the identification of novel transcription factors and cell surface proteins which have been confirmed by independent groups. This both demonstrates the remarkable fate specificity of ESC-derived mDA neuron and outlines the sequential stage-specific reporter line paradigm for in vivo gene discovery. Finally, preliminary data indicate that two surface markers identified may be used for in vivo visualization of mDA neurons and the purification of hESC-derived mDA neurons.

W-2055

SCALABLE PASSAGING OF HUMAN PLURIPOTENT STEM CELLS

Ying Nie, Patrick Walsh, Diana Clarke, Jon Rowley, Thomas Fellner
Lonza Walkersville, Inc., Walkersville, MD, United States

Human pluripotent stem cells (hPSCs) hold great promise for the development of cell replacement therapies. However, for this promise to be realized numerous challenges need to be overcome; one of which is production of clinical-grade cells in a cost-effective manner. A critical but commonly overlooked step is the subcultivation of the cells. Current methods used to subculture hPSCs are incompatible with clinical manufacturing processes because they are labor intensive and result in reduced cell viabilities. To improve on these methods, we set out to develop a cGMP-compliant, non-enzymatic passaging reagent that requires minimal manual manipulation of cells. This hypertonic sodium citrate-based solution promotes multicellular colony detachment and high post-detachment cell viability. Treatment of hESC cultures with this passaging solution results in a post-detachment cell viability of 97%. Comparatively, conventional methods such as colony scraping, Collagenase IV or Dispase treatment exhibit significantly lower cell viabilities of 27, 58 and 67% in StemPro® and 31%, 47% and 53% in mTeSR™1, respectively. The increase in viable cells achieved through the use of the hypertonic sodium citrate-based solution at each passage significantly reduces the time required to generate sufficient quantities of hPSCs for clinical-scale production. The total number of cells produced from a starting population of $2x10^5$ hPSCs utilizing this passaging process exceeds $2x10^{12}$ cells. We also demonstrated that hPSCs continuously sub-cultivated with the hypertonic citrate solution for over 25 passages retain a normal karyotype and co-express the classic subset of markers indicative of hPSC pluripotency: Oct4, SSEA4, Tra-1-60, Nanog and Tra-1-81. We believe this streamlined passaging process offers a new method suitable for the cultivation of high-quality hPSCs in the research laboratory and clinical manufacturing suite.

W-2056

PATENTING LIFE, US SUPREME COURT'S DECISION ON GENE PATENTS AND ITS POTENTIAL IMPACTS ON STEM CELL RESEARCH

Maude L. Cuchiara, Kirstin RW Matthews
Baker Institute for Public Policy, Rice University, Houston, TX, United States

Patenting Life, US Supreme Court's Decision on Gene Patents and its Potential Impacts on Stem Cell Research
Kirstin RW Matthews* and Maude L Cuchiara*
*James A. Baker III Institute for Public Policy, Rice University
In 1980, the landmark case *Diamond v. Chakrabarty* guaranteed the ability to patent some forms of life. Researchers fought to patent a bacterium that had been genetically modified to digest oil, and ultimately, the court ruled that "anything under the sun made by man" was patentable. This decision led to the patenting of mice strains, human stem cells, and other living organisms or their components, including genes. In 1997 and 1998, Myriad Genetics was granted patents for two genes—BRCA1 and BRCA2—which are implicated in hereditary breast and ovarian cancer. Due to the patent, Myriad is currently the only company that can conduct diagnostic testing for these genes. The patenting of the BRCA genes launched a raucous debate about the ability to patent life: how do we distinguish between what is simply discovered and what is truly "made by man"?
The American Civil Liberties Union (ACLU) and the Association for Molecular Pathology challenged the gene patents, and the US Supreme Court selected the case for its 2013 docket. The court will rule on one of Myriad Genetics' claims: the patentability of human genes. The decision made in this case will affect the patentability of other biological "inventions" such as human embryonic stem cells (hESCs). In the US, hESC patents are almost exclusively held by the Wisconsin Alumni Research Fund (WARF). Many researchers in the field opposed these patents for a similar rationale as gene patent opponents: hESCs were discovered, not created or made. As a result, in 2006, the Foundation for Taxpayer and Consumer Rights and the Public Patent Foundation asked the US Patent and Trademark Office (USPTO) to revoke the patent. The USPTO ultimately validated the patents but limited their breadth and mandated a relaxation in licensing procedures. If the Supreme Court rules that gene patents are invalid, the ability to patent hESCs would likely be challenged and possibly revoked.
Here we will describe the progression of the Myriad Genetics case as well as compare the legal and scientific rationale for and against gene patents to that of hESC patents. We will then discuss the arguments made by both sides to the Supreme Court and speculate on how these arguments and each possible Supreme Court ruling (if a ruling has not been released) apply to the patenting of hESCs and surrounding technologies. We will also compare US and EU policies on similar technologies and elaborate on the problems associated with the judicial system ruling on regulations of highly scientific material. Finally, we will reflect on the appropriateness of these types of patents – comparing the BRCA gene and WARF stem cell patents.

W-2057

EXPANDING BIOLOGICAL PATHWAY MODELS WITH FUNCTIONAL GENOMICS DATA IMPROVES COMPUTATIONAL PATHWAY ANALYSIS OF STEM CELL DIFFERENTIATION

Daniel Carlin, Joshua Stuart
Biomolecular Engineering, University of California- Santa Cruz, Santa Cruz, CA, United States

The high throughput analysis of the transcriptome has become a central tool in stem cell biology. Although some important transcription factors have been elucidated, little is known about the pathways involved in differentiation from a systemic point of view. We have developed an integrated analysis method called PARADIGM for the inference of pathways activated in cancer from the analysis of genomics data sets. The method has successfully identified aberrant gene hubs in many tumor types including glioblastoma, ovarian, breast, and kidney cancer. To investigate stem cell processes, we have extended the pathway database utilized by PARADIGM by incorporating predicted interactions from the analysis of functional genomics datasets including ChIP-Seq, co-expression data across a large number of tissue types, conservation of binding sites, protein-protein interactions, and miR target predictions. The newly incorporated evidence is organized into "tiers" that reflect the amount of independent data sources support the existence of each interaction. By adding the highest tier of most supported interactions to our pathway database, we have been able to provide suggestions for novel gene interactions that are important in stem cell differentiation. We applied this new, expanded pathway database to the meta-analysis of publicly available stem cell datasets to provide a robust pathway view of commonly performed differentiation assays. Several of the active gene clusters are common to cancer pathways and suggest links between stemness and cancer, and give clues to the treatment of cancers. We also applied the expanded pathway database to a cortical neuron differentiation RNA-seq time course. By isolating the biological entities most active in the differentiation course, we are able to provide gene candidates for inducing and repressing cortical neural differentiation.

W-2058

ENDODERM DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS BY CELL PATTERNING.

Takuya Okazaki[1], Masakazu Machida[2], Ryo Tamoto[2], Yuichi Tanaka[1], Katsunori Tsuchiya[1], Yuji Shimizu[1], Hidenori Akutsu[2], Akihiro Umezawa[2]
[1]Life Science Laboratory, Dai Nippon Printing Co., Ltd., Tokyo, Japan, [2]Department of Reproductive Biology, National Center for Child Health and Development, Tokyo, Japan

Human pluripotent stem cells (hPSCs) have the potential to be used in many applications due to their ability to differentiate into cells of any of the three germ layers (ectoderm, endoderm, and mesoderm). When guiding differentiation, embryoid bodies are made in culture solution containing differentiation-inducing factors and subsequently maintained for a fixed period with adhesive cultivation. However, high efficiencient directed differentiation is technically challenging and currently a bottleneck in the field. Differentiation of hPSCs to a mature cell type typically requires serial cell culture steps with sequential addition of extrinsic factors including growth factors and cytokines. Testing a significant number of such differentiation protocols is therefore very labor intensive and time consuming and limits the development of optimized methods. In order to solve such problems, we have developed cell culture materials.
Generally, cells respond differently to substrates of varying stiffness of culture plates. In the current work, we set up several conditions of cell patterning using micro-fabrication technique and investigate the effect of microstructural features on the differentiation of hPSCs. Multiple gene expression analysis of cultured hPSCs on various diameter circle patterned substrates by qRT-PCR using a TaqMan Array revealed that smaller pattering induced endodermal markers expression including Afp, Foxa2, Gata6 and Isl1. This analysis reveals the sensitivity of cellular phenotype commitment and differentiation patterning responding microstructural features. It would be used to help guide the design of scaffolds with specific properties for tissue engineering applications using hPSCs.

W-2061

SYNTHEMAX II-SC, A NOVEL SYNTHETIC SURFACE FOR PLURIPOTENT STEM CELL CULTURE.

Zara K. Melkoumian, Jerry Zhou, David Henry, Paula Dolley-Sonneville, Jennifer Weber
Life Sciences Development, Corning Inc, Corning, NY, United States

Scalable, reproducible, low cost and regulatory-friendly technologies must be developed to enable clinical use of pluripotent stem cell (PSC)-based therapeutics. PSC culture methods use complex, animal-derived products, such as mouse feeder layers and mouse tumor extracts or human-derived biological substances to coat culture vessels. Most of these materials are costly, of limited scalability, have high batch to batch variability and are a potential source of adventitious agents. For clinical applications of PSC-based therapeutics it is highly desirable to have defined, scalable culture systems for production of cells suitable for clinical use.
In this study we describe a fully synthetic substrate, Synthemax II-SC, for the culture of PSCs. The substrate is comprised of an RGD-containing peptide sequence derived from the active domain of the vitronectin protein linked to an acrylate polymer. The surface coating protocol with Synthemax II-SC is very simple and consists of dissolving the powder substrate in water, dispensing and incubating in a culture vessel for 1-2 hours. Unlike biological coatings, Synthemax II-SC is very stable, and pre-coated vessels can be stored for up to 2 months.
We have demonstrated a multi-passage expansion of hiPSCs and hESCs on Synthemax II-SC coated plates in mTeSR1 defined medium. Importantly, cells maintained typical morphology, consistent doubling time, expression of pluripotency markers (Oct4 and SSEA4), normal karyotype and the potential to differentiate into cell types of all three germ layers after long-term culture on Synthemax II-SC coated plates.
In combination with defined medium, Synthemax II-SC provides a complete culture system for both research applications and scalable manufacturing of PSCs.

W-2062

PERFUSED MICROPLATE FOR CONTROLLED STEM CELLS DIFFERENTIATION

Kaitlyn Matias, Vasiliy Goral, Po Ki Yuen, Florence Verrier
Biochemical Technologies, Corning Inc, Corning, NY, United States

Human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs) hold great potential for cell therapy, drug efficacy research, toxicity screens and predictive disease modeling. This is mainly due to two distinct properties: (i) they can self-renew indefinitely and (ii) they can potentially generate all cell types in the human body. The challenge in using hESCs or progenitor stem cells for various applications has been to direct the wide differentiation potential toward the derivation of a specific cell fate. hESCs or progenitor neural stem cells can be differentiated *in vitro* into neural progenitor cells, neurons, oligodendrocytes and astrocytes. However, there is still a lack of efficient and robust protocols to obtain high percentage of differentiated neuronal cells that show good maturity and characteristics of *in vivo*-like functional cells.
Controlling the cell microenvironment has become crucial in the stem cell field where scientists are trying to differentiate or reprogram cells typically by overcoming these signals via adding their own exogenous factors. These exogenous factors are often added at saturating concentrations, blanketing the cells to activate specific pathways to produce a desired outcome (e.g. specific phenotype). However, the constant din of cell communications is still present, causing alternate and perhaps opposing pathways to be stimulated. This constant cell secretion in conventional static culture limits therefore the development of new methods to elicit a specific phenotype.
Here we describe a new method of culture (passively perfused cell culture) creating a dynamic microenvironment allowing better stem cell growth and stem cell differentiation relative to conventional static culture. Specifically, we demonstrated that passively perfused cell culture improved stem cell performance (increased growth rate) compared to conventional static stem cell culture, without altering their self-renewal property. We also demonstrated a substantive improvement of the differentiation of neural progenitor stem cells into astrocytes and neurons. This method of culture is also very attractive as it does not require any daily medium change, reducing substantially the labor of culture. Overall, the passively perfused microplate can be used for spatiotemporal control over the stem-cell microenvironment, so that the ideal *ex vivo* niche for cell survival or differentiation can be defined quantitatively and in high throughput.

W-2063

NON-BIASED COMPARATIVE ANALYSES OF PIGGYBAC, TOL2, AND SLEEPING BEAUTY TRANSPOSON SYSTEMS BY A NON-DRUG SELECTION PLATFORM IN HEK293

Yaa-Jyuhn James Meir, Sareina Chiung-Yuan Wu
Chang Gung University, Kwei-Shan, Taiwan

Three non-viral DNA transposon systems, *Tol2*, *piggyBac*, and *Sleeping Beauty*, have gained momentum as genetic tools for mammalian genome manipulations. Previous reports have provided guidance for choosing the most suitable transposon to meet individual experimental needs by comparing *piggyBac*, *Tol2, and Sleeping Beauty* in terms of their activity and genome-wide targeting profiles. However, all of these studies focused exclusively on transposon targeting events that result in transgene expression, and consequently under-represent practical situations encountered in fundamental and translational/clinical research where it is also necessary to analyze transposition events that result in transgene silencing.
In this study, we exploited a novel non-drug selection platform to gain a non-biased and more insightful comparison of the three transposon systems in HEK293 cells. We analyzed chromosomal targeting activity, transgene expression rate, and the genome-wide target profile of transposon-integrated clones with or without transgene expression. We found that the most favorable system to uncover genes that are involved in key differentiation processes but are epigenetically silenced at the time of targeting is *piggyBac*, due to the following characteristics: (1) its extremely high chromosomal targeting rate (~ 94%) in a drug-free setting, (2) its high transgene expression rate, (3) its ability to access inactive chromosomal regions, and (4) its preferential intragenic targeting. The first two characters along with its lowest transgene silenced rate and its unique amenability for molecular engineering to achieve site-specific therapeutic gene targeting make *piggyBac* the most promising transposon among these three for gene therapy.

W-2064

ACTIVATED PLATELET SUPERNATANT CAN AUGMENTED THE ANGIOGENIC POTENTIAL OF PERIPHERAL BLOOD STEM CELLS IN STEM CELL THERAPY.

Jin-A Kang, Jin Hur, Ji-Yeon Yun, Jae-Il Choi, Young-Eun Choi, Ju-Young Kim, Jeehoon Kang, Ji-Hoon Hong, Jung-Kyu Han, Hyun-Jai Cho, Young-Bae Park, Hyo-Soo Kim
Seoul national university, Seoul, Korea, Republic of

Despite the progress in stem cell therapy, efficacy of tissue repair is still modest in ischemic diseases. Although platelets are known to play a role in hemostasis, they also promote angiogenesis by releasing various cytokines. We investigated the improvement of stem cell therapy, by priming granulocyte-colony stimulating factor (G-CSF) mobilized peripheral blood stem cells (mobPBSCs) with ‘activated platelet supernatants (APS)’.
Healthy volunteers underwent daily subcutaneous injections of G-CSF for 3 days, and mobPBSCs were isolated while APS was collected by thrombin activation of the platelet rich plasma. APS contained various cytokines such as IL-8, IL-17, PDGF and VEGF. APS-primed mobPBSCs increased expression of genes to support angiogenesis and mobPBSC differentiation was polarized toward CD14++/CD16+, so called pro-angiogenic monocytes, with an increase of cell surface integrins. To check the paracrine effect of APS-priming, primed mobPBSCs were cultured for 36 hours. The supernatants of APS-primed mobPBSCs could augment proliferation and formation of a capillary network formation in human umbilical vein endothelial cells. In vivo transplantation of APS-primed mobPBSCs into athymic mice ischemic limb and Matrigel plugs elicited enhanced vessel differentiation, which improved tissue repair. Safety analysis of APS primed mobPBSCs was proven by the increased platelet activity when primed mobPBSCs were mixed in whole blood, which was decreased when pretreated with aspirin.
Collectively, our data identify that APS priming can enhanced the angiogenic potential of mobPBSCs, which can be used as a novel method in increasing efficacy of stem cell therapy.

W-2071

DISCOVERY OF A NOVEL PLURIPOTENCY MARKER BC2LCN USING LECTIN MICROARRAY

Hiroaki Tateno, Yasuko Onuma, Yuzuru Itou, Makoto Asashima, Jun Hirabayashi
AIST, Tsukuba, Japan

Induced pluripotent stem cells (iPSCs) can now be produced from various somatic cell (SC) lines by ectopic expression of the four transcription factors Oct4, Sox2, c-Myc, and Klf4. Although the procedure has demonstrated to induce global change in gene and microRNA expressions, and even epigenetic modifications, it remains largely unknown how this transcription factor-induced reprogramming affects the total glycan repertoire expressed on the cells. Here we performed comprehensive glycome analysis of a large set of hiPSCs (114 cell types) and hESCs (9 cell types) using lectin microarray. In unsupervised cluster analysis of the results obtained by lectin microarray, both undifferentiated iPSCs and ESCs were clustered as one large group. However, they were clearly separated from the group of differentiated SCs, while all of the four SCs had apparently distinct glycome profiles from one another. This observation demonstrates that SCs with originally distinct glycan profiles have acquired those similar to ESCs upon induction of pluripotency. Thirty-eight lectins discriminating between SCs and iPSCs/ESCs were statistically selected, and characteristic features of the pluripotent state were obtained at the level of the cellular glycome: these include increased α2-6sialylation, α1-2fucosylation, and type1 N-acetyllactosamine (LacNAc), while corresponding decrease in α2-3sialylation, type2 LacNAc, and highly-branched N-glycans was observed. The expression profiles of the relevant glycosyltransferase genes agreed well with the results obtained by lectin microarray. Through the analysis, we identified a lectin rBC2LCN binds only undifferentiated iPSCs/ESCs but never differentiated SCs. rBC2LCN worked as a specific probe for staining and sorting of iPSCs and ESCs. Even live colonies of iPSCs and ESCs could be visualized just by the addition of fluorescein-labeled rBC2LCN into cell culture media. No effect on the properties of iPSCs and ESCs by rBC2LCN could be confirmed by DNA microarray, indicating that rBC2LCN is a suitable probe for live cell monitoring of human pluripotent stem cells during cell culture. Cell surface glycoprotein ligands of rBC2LCN were also examined. Podocalyxin, a hyperglycosylated transmembrane protein, was found to be a predominant one. Furthermore, rBC2LCN exhibited significant affinity to a mucin-type O-glycan comprising an H type3 structure prepared from human 201B7 iPSCs, suggesting that H type3 is a novel pluripotency marker. rBC2LCN can be expressed at high levels in a soluble form in the cytoplasm of Escherichia coli (80 mg/L) and can be purified to homogeneity in a one-step sugar-immobilized affinity chromatography approach. Thus, rBC2LCN, unlike antibody, could serve as a novel type of detection reagent of pluripotent stem cells, which is cost-effective and easy to produce in a large amount.

W-2072

NOVEL MACROCARRIERS FOR IMPROVED HUMAN PLURIPOTENT STEM CELL EXPANSION IN STIRRED TANK BIOREACTORS.

Brian M. Davis[1], Evelina R. Loghin[1], Andrew A. Burns[2], Reginald D. Smith[1], Scott M. Miller[2], Kenneth R. Conway[3], Martin J. Brown[1], David G. Gascoyne[2]

[A]Life Sciences and Diagnostics, [B]Nanostructures and Surfaces Laboratory, [C]High Energy Physics Laboratory, [1]GE Global Research, Niskayuna, NY, United States

A need for large scale pluripotent stem cell culture is emerging for applications in pluripotent stem cell banking (e.g., for induced pluripotent stem cells), commercial production of cells (e.g., GE's Cytiva™ cardiomyocytes), and cell expansion for clinical trials. Advances in feeder-free embryonic stem cell culture have enabled large scale cell expansion, which is conventionally performed in flasks, but maintenance of large numbers of flasks is labor intensive, space prohibitive and poses concerns that individual flasks may exhibit phenotypic drift. Therefore, new approaches for scaling up stem cell culture are required. The research presented here describes human embryonic stem cell expansion in stirred tank bioreactors using a novel macrocarrier developed at GE Global Research. Conventional microcarriers are 150 to 250 microns in diameter; in comparison, GE's proprietary macrocarriers are ~6 mm in diameter and support the growth of more than 150,000 human embryonic stem cells per carrier. The density of the macrocarrier material easily promotes their fluidization with the generation of minimal shear forces under stir conditions, and rapid sedimentation in the absence of agitation. Four embryonic stem cell lines (CHB10 [from Dr. George Daley, Children's Hospital Boston], CT2 [from University of Connecticut Health Center], and H1 and H7 [both from Geron Corp.]) were expanded on GE's macrocarriers. The carriers were coated with different matrices that support embryonic stem cell attachment and growth including Matrigel™, Laminin 521 and Synthemax II™.

Cells were seeded onto to the macrocarriers in stirred tank bioreactors and expanded for 3 to 5 days prior to enzymatic passaging. The large size of the macrocarriers facilitated the gentle separation of cells from carriers during enzymatic cell recovery. The human embryonic stem cells exhibited similar doubling times when cultured on macrocarriers in stirred tank bioreactors as when cultured in 6-well plates and were morphologically similar to colonies grown in static culture. All four embryronic stem cell lines were maintained for 10 or more serial passages on GE's macrocarriers in stirred tank bioreactors. At the conclusion of macrocarrier culture, cells were analyzed for the pluripotency markers Oct4 and SSEA4, karyotype, embryoid body and teratoma formation. Cells recovered from the macrocarriers and replated onto 6 well plates retained cell morphology and expansion rates equivalent to the parental cells. Comparison cultures grown on commercial microcarriers (Cytodex 1^{TM}, Microhex^{TM} and Hillex II^{TM}) exhibited carrier-to-carrier bridging resulting in large clusters, three-dimensional colony growth, and reduced cell viability and recovery. In conclusion, four hESC cell lines were successfully expanded for 10 or more passages on novel shear protected macrocarriers while maintaining pluripotency in stirred tank bioreactors. This novel macrocarrier design solves many of the challenges that occur when culturing pluripotent cells on traditional microcarriers by providing shear projection, allowing easy separation of the carriers from cells during passaging, and preventing the formation of cell-carrier aggregates.

W-2073

AN EFFECTIVE VERIFICATION OF ANTIFREEZE POLYAMINO-ACID FOR A SLOW CRYOPRESERVATION OF HUMAN IPS CELL AND ES CELL

AKEMI OTA[1], Kazuaki Matsumura[3], Hirofumi Suemori[2], Shoichiro Sumi[1], Suong-Hyu Hyon[4]
[A]Department of Organ Reconstruction, Field of Clinical Application, [B]Department of Embryonic Stem Cell Research, [1]Institute for Frontier Medical, Kyoto University, Kyoto, Japan, [2]Department of Materials Science New Materials Design and Synthesis, School of Materials Science, Japan Advanced Institute of Science and Technology, Nomi, Japan, [3]Center for Fiber and Textile Science, Kyoto Institute of Technology, Kyoto, Japan

[Objective] The cryopreservation of human iPS cells using slow-freezing after the single-cell passaging is better than that using vitrification of aggregated cells of dissected colonies, because huge volume of iPS cells is necessary for the clinical application. Furthermore, the single-cell passaging enables the differentiation experiment or the gene transfer into iPS cells easier than that of aggregated cells on feeder layer. Dimethyl sulfoxide (DMSO) is a superior cryoprotective agent, but it should be decreased as possible because of its higher cytotoxicity. In this study we developed the cryopreservation solution using carboxylated ε-poly-L-lysine (COOH-PLL), and investigated its effectivity after freezing for one month.
[Method] The cryopreservation solution was contained 3%COOH-PLL, recombinant human albumin and DMSO, whose concentration decreased as possible in DMEM/F12 medium. Single cell suspension of human iPS cells in the cryopreservation solution was stored at -80^{O}C for one month. After thawing the iPS cells and ES cells were cultured on the matrigel-coated plate with SNL-conditioned medium containing bFGF for four passages, and then tested for proliferation ability and multipotency.
[Results] Human iPS cells and ES cells were cultured in good adherence. The cell growth and multipotency were kept after four passages. The cryopreservation solution with 1% or 2% COOH-PLL decreased total colony numbers at AP staining. The concentration of DMSO was able to be decreased down to 6%. Adding the rock inhibitor, Y-27632, to the cryopreservation solution showed little effect for cryopreservation.
[Conclusion] We investigated the ability of cryopreservation solution using COOH-PLL at the stock period of one month and showed its effectivity. This solution is xeno-free and enough to be used for the clinical application. To the goal of future research, this cryopreservation solution will be developed for longer and huge storage.

W-2074

NANOCRYSTAL TRACKING OF A SLOW CYCLING STEM-LIKE SUBPOPULATION IN HUMAN ENDOMETRIUM

Lina N. Xiang, **Rachel W.S Chan**, Ernest H.Y Ng, William S.B Yeung

Obstetrics & Gynaecology, University of Hong Kong, Hong Kong, Hong Kong

Introduction: Human endometrium displays cyclical regeneration and tissue breakdown throughout a woman's reproductive life. Putative stem cells within the mucosal lining have been shown to be responsible for the remarkable regenerative capacity. A key feature of somatic stem cell is their quiescent state. However it remains unclear whether slow cycling cells exist in human endometrium. The objective of this study was to establish a novel in vitro stem cell assay employing nanoparticle tracking to isolate putative slow-cycling cells in endometrial stromal cultures.
Methods: Single human endometrial stromal cells obtained from women undergoing hysterectomy were labeled with fluorescent nanocrystals followed by a chase of 21 days in culture. Two population of stromal cells: slow-cycling (fluorescent persistent cell; FPC) and rapidly proliferating (non-fluorescent persistent cells; non-FPC) cells were isolated using fluorescent activator cell sorter. The stem cell properties of FPC and non-FPC were compared through the use of colony forming assay, serial subcloning, total cell output and in vitro differentiation assay. Molecular characterization of the two populations was assessed using real-time polymerase chain reaction with pluripotent (NANOG, OCT-4, SOX-2) and self-renewal (BMI-1) genes. The phenotypic expression of endometrial mesenchymal markers (CD146/CD140b, W5C5) on FPC and non-FPC was also examined using flow cytometry.
Results: Maximum fluorescent labeling of endometrial stromal cells (100%) was achieved 24 hours after primary cell culture. A minority (0.90 ± 0.09%, n=19) of FPC possessed the nanocrystals after long term culture (day 21). Assessment of stem cell characteristics revealed stromal FPC displayed a significantly higher colony forming ability and underwent more rounds of self-renewal than non-FPC. The multipotency of stromal FPC to differentiate into the mesenchymal lineages upon exposure to adipogenic, osteogenic, myogenic and chondrogenic induction media was significantly higher than non-FPC. Clonally derived FPC expressed mRNA of pluripotent and self-renewal (NANOG, OCT-4, SOX-2, and BMI-1) genes. Furthermore, there was significantly higher percentage of cells within the FPC population expressing of endometrial mesenchymal stromal cell markers (CD146+/CD140b+ or W5C5+).
Conclusion: We have established a novel in vitro endometrial stem cell assay in which putative slow cycling human endometrial stromal cells can be identified through nanoparticle tracking. Using this assay, we demonstrated that FPC share common somatic stem cells properties, in particular that of mesenchymal origin.
Acknowledgement: This study was supported by Research Grant Council (HKU 7822/09M).

W-2081

BIOREACTOR-BASED EXPANSION OF MESENCHYMAL STEM CELLS ON NOVEL SHEAR-PROTECTED CARRIERS

Vandana Keskar[1], Joanne F. Smith[1], Reginald Donovan Smith[1], Zhipeng Zhang[2], Andrew A. Burns[3], Scott Michael Miller[4], Martin James Brown[1], David Gilles Gascoyne[3]
[A]Life Sciences & Diagnostics, [B]Machines Laboratory, [C]Nanostructures and Surfaces Laboratory, [D]Engineered Materials, Chemistry & Physics, [1]GE Global Research, Niskayuna, NY, United States

Mesenchymal stem cells (MSCs) are being researched for multiple therapeutic applications due to their immunomodulatory properties and tri-lineage differentiation potential. Clinical trials target MSCs for cell therapy and regenerative medicine applications such as graft versus host disease, critical limb ischemia, acute myocardial infarction and osteoarthritis. High cell doses (up to 5 MM cells/kg body weight) necessitate fast and reproducible *in vitro* MSC expansion techniques. Adherent cell expansion is often carried out on planar static formats. These methods involve handling of many pieces of cultureware, clean rooms and multiple manual operations which are inefficient and increase the risk of contamination. Bioreactors with microcarriers have the potential to address the above concerns for adherent cell expansion. However, cell numbers generated through these techniques are less than satisfactory due to the challenges in growing shear sensitive MSCs in fluidized carrier systems. Other challenges with traditional microcarriers for MSC expansion are difficulty in visualizing cells on the carriers, ease of harvest and cell separation from the microcarriers.
To address the above concerns and provide a novel platform for adherent cell expansion in bioreactors we developed polystyrene(PS)-based macro-carriers. The 6mm hexagonal carriers are made of plasma-treated, medical grade polystyrene and provides cells with a growth surface chemically similar to T-flasks. Relief features on the

carriers provide shear protection to the adherent cells thus enabling use of the carriers in bioreactors. The carrier density and size are designed to enable easy fluidization in dynamic conditions. Multiple lots of commercially available bone marrow-derived MSCs (p2) were successfully expanded on the PS carriers in spinner flasks. Initial cell attachment on the carriers in dynamic conditions was similar to attachment on static T-flasks. The expansion protocol was optimized for impeller rpm, agitation on/off times and feed schedules to obtain T-flasks comparable cell densities at harvest. The doubling times for MSCs grown in the spinner flasks was also comparable to static T-flask controls. No additional surface coatings were required to support cell growth in serum-based medium. For serum-free culture of MSCs, the carriers were pre-coated with cell-attachment matrices with the same protocols as for T-flasks. Unlike most commercial microcarriers where aggregation of the carriers is observed at high cell densities, no aggregation of the polystyrene carriers was observed even at cell densities of 30,000 cells/cm^2. The native spindle-shaped morphology of cells grown on the carriers could be viewed under the brightfield microscope. Cell separation from the carriers at harvest was complete with >95% viability using traditional enzymatic dissociation technique and did not necessitate use of cell filters or any additional cell separation equipment due to the large size of the carriers relative to the cells. The MSCs harvested post bioreactor expansion retained their tri-lineage differentiation potential and cell surface markers (CD105+, CD90+ & CD73+). This novel carrier based bioreactor system has the potential to enable MSC expansion in a closed, automated and scalable manner thus reducing labor and contamination risks associated with static planar cultures.

W-2082

REDUCING MICROBIAL CONTAMINATION IN STEM CELL CULTURE THROUGH H2O2 DECONTAMINATION TECHNOLOGY - INCREASED EFFICIENCY AND EFFICACY WITH SIGNIFICANTLY REDUCED DOWNTIME FOR STEM CELL CULTURE

Deepak M. Mistry[1], Nehal Kothari[2], Kondala Atkuri[3]

[A]Marketing & Strategic Development, [B]Marketing & Strategic Application, [1]Panasonic Healthcare Company of North America, Wood Dale, IL, United States, [2]Genetics, Stanford University, Stanford, CA, United States

The most common form of contamination in stem cell products for cell therapy and regenerative medicine are Bacteria (including *Mycoplasma*), yeast and fungi. Recent studies have found that Mycoplasma contamination rate in these stem cell products was between 4% to as high as 22% with overall cell culture contamination of up to 12% or even higher in some cases. Of all the likely sources, the cell culture incubator has often been considered one of the most probable sources of biological contamination. Many incubator manufacturers have addressed this issue using different types of sterilization technologies. A study was undertaken to determine the effectiveness and efficiency of three industry-leading incubators that utilize different sterilization techniques: Brand P with the H_2O_2 decontamination technology that requires 2 hours for its cycle with around 15 minutes of prep time, Brand A with a dry heat sterilization method that takes 10-12 hours for the complete process, and Brand B with a moist heat sterilization method that requires up to 25 hours from start to finish. Five different microorganisms, generally considered the most common contaminants in a cell culture environment, were tested in this study: *Candida albicans, Acholeplasma laidlawii, Mycoplasma orale, Bacillus subtilis, and Staphylococcus aureus*. At a specific CFU, each organism was placed on both upper and lower shelves of the incubators, which were then allowed to go through their individual sterilization cycle. A time control coupon was also run simultaneously for the same amount of time as the sterilization cycle. Each of the three cell culture incubators showed complete bioburden reduction for each organism. However, the H_2O_2 decontamination technology offered complete organism elimination in 2 hours, as compared to 10-12 hours and 25 hours for Dry heat and Moist heat sterilization technology, respectively. In addition, a significant reduction in viability was observed for *Candida albicans, Acholeplasma laidlawii and Mycoplasma orale,* when each time control was compared. The H_2O_2 decontamination technology effectively showed a greater bioburden reduction than the heat sterilization techniques, when the 2-hour time control was compared to the 10-12 hour and 25 hour time controls. This validates the effectiveness with which the H_2O_2 technology decontaminates the chamber. For *Staphylococcus aureus and Bacillus subtilis*, an increase in viability was observed over time

when compared to time controls. However, the H_2O_2 decontamination technology efficiently eliminated all of the bioburden in a considerably shorter span of time without significantly increasing the original concentration of the organisms. The 2-hour uptime advantage offered by the H_2O_2 decontamination technology with 100 % kill rate and at least a 6 log reduction for all the organisms tested, lead to increased productivity in a research or GMP environment.

W-2083

NANOPORE PATTERN-INDUCED DEFINITIVE ENDODERMAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS.

Jong Hyun Kim[1], Jiyou Han[1], Su Yoen An[1], Yu Jin Jang[1], Jeong Sang Son[1], Gyunggyu Lee[2], Ji Young Park[1], Hyung Woo Kim[3], Kyoung Je Cha[4], Dong Sung Kim[4], Jong-Hoon Kim[1]

[A]Laboratory of Stem Cells and Tissue Regeneration, College of Life Sciences and Biotechnology, [B]Department of Stem Cells and Tissue Regeneration, College of Life Sciences and Biotechnology, [1]Korea University, Seoul, Korea, Republic of, [2]Department of Mechanical Engineering, POSTECH, San 31, Hyoja-dong, Nam-gu, Pohang, Gyeongbuk, Korea, Republic of, [3]Department of Mechanical Engineering, POSTECH, San31, Hyoja-dong, Nam-gu, Pohang, Gyeongbuk, Korea, Republic of

Human embryonic stem cells (hESCs) have been proposed as a promising candidate for tissue regenerative therapies to cure various diseases, including nerve, heart liver, and pancreas. Therefore, intensive studies have been carried out to screen effective and cost-beneficial inducing agents including growth factors, cytokines, and extracellular matrices to differentiate hESCs into a special linage of somatic cells. In general, physical extracellular microenvironment provides crucial inducing factors to stem cells themselves and surrounding extracellular substrates. In this study, we describe a direct, simple, and effective differentiation of hESCs into definitive endodermal cells using only a nanopore pattern chip without any growth factors and cytokines. hESCs were dissociated into single cells and grown on the nanopore pattern chip (pore size; 200nm) in RPMI medium in the absence of any growth factors. Real-time PCR, immunochemistry, and proteome array data showed that the nanopore pattern chip significantly increased endodermal differentiation of hESC. Compared to conventional culture dishes, the number of SOX17-positive cells was significantly increased by differentiating hESC on the nanopore pattern (approximately 2-fold, $P<0.05$). The SOX17-positive cells coexpressed CXCR4 as well as FOXA2, which are typical markers of the definitive endoderm. Expression of endoderm-associated proteins was increased by growing on the nanopattern chip. Consequently, the nanopore pattern chip developed in this study could be a new effective and cost-beneficial tool for the endodermal differentiation of hESCs.
This work was supported by the Industrial Core Technolgy Development Program (No.10041913) funded by the Ministry of Knowledge Economy.

W-2084

Whole-cell mass spectrometry profiling combined with artificial intelligence as a novel tool for fingerprinting of hESCs

Petr Vaňhara[1], Lukáš Kučera[1], Filipo Amato[2], Eladia María Peña-Méndez[3], Milan Ešner[1], Josef Havel[2], Ales Hampl[1]

[A]Department of Histology and Embryology, [B]Department of Chemistry, [1]Masaryk University, Brno, Czech Republic, [2]Department of Analytical Chemistry, Nutrition and Food Science, University of La Laguna, La Laguna, Spain

There is an ongoing need for a robust, feasible and sensitive method for determining cell identity and/or revealing possible phenotype perturbations. The molecular, genetic and/or light-microscopy analyses fail in case of small and subtle but critical changes arising in cultured cells. General bio-analytical methods, e.g. matrix-assisted laser desorption/ionization - time of flight mass spectrometry (MALDI-TOF MS) have manifold applications in classical analytical and structural chemistry. Due to its inherent principal universality, this method became attractive beyond

chemistry and has been adapted also for characterization of complex biological samples. However, mass spectra generated from ionized molecules desorbed from the whole cells are rather complex and usually reflect not only the biological variability but also variety of other factors, e.g. experimental conditions. Artificial neural networks (ANNs) represent a non-linear mathematical model resembling a brain neural architecture and possessing "learning" and "generalization" abilities. Such sophisticated mathematical analysis reduces unwanted inconstancy and can identify hidden patterns in mass spectra.
As a proof of the principle, we first aimed to discriminate between biologically distinct cell types, human embryonic cells (hESCs) and mouse embryonic fibroblasts (MEFs). The hESCs and MEFs were cultured under standard conditions, harvested by scraping, washed in inorganic buffers and mixed with acidified matrix without corruption of cellular integrity. Several organic matrices as well as sample deposition methods were tested. The MALDI-TOF MS was then performed in several mass ranges, with varying detector mode and laser power. The mass spectra reached high-level of reproducibility in the range of approx. 6000-16000 m/z, equivalent to molecular weight approx. 6-16 kDa. We were able to identify sample specific peaks and to perform cluster/factor analysis. Despite the successful classification of all analyzed samples, the factor analysis does not allow to perform nonlinear clustering of samples. Using ANNs we were able to compensate for nonlinearities in complex data (e.g. biological and instrumental variability). The robust database of whole-cell mass spectra was build and served as input for feed-forward neural network. After training of the ANN, we achieved 100% discrimination between hESCs and MEFs. We then tested whether this approach can discriminate between morphologically uniform, yet innerly different hESCs. The hESCs were stimulated with 0,1µM retinoic acid (RA) for only 10 hours or left untreated and then processed with whole-cell MALDI-TOF MS. The correctly trained ANNs were able to reach significantly high level of discrimination while the cluster and factor analysis did not identify differences. In summary, we developed and optimized a simple, robust and efficient cell authentication tool enabling routine use in clinical grade cell cultures.
This work is supported from Ministry of Education, Youth and Sports of the Czech Republic (Projects MSM0021622411, MSM0021627501, MSM0021622430, CZ.1.05/1.1.00/02.0123, CEPLANT CZ.1.05/2.1.00/03.0086, and HistoPARK CZ.1.07/2.3.00/20.0185) and the Czech Science Foundation (Projects 104/08/0229, 202/07/1669).

W-2091

A NONINVASIVE METHOD FOR COUNTING HUMAN PLURIPOTENT STEM CELL NUMBERS BY LIVE CELL IMAGING

Mika Suga[1], Hiroaki Kii[2], Takayuki Uozumi[2], Yasujiro Kiyota[2], Miho K. Furue[1]
[1]Dept of Disease Bioresources Research, Laboratory of Stem Cell Cultures, National Inst of Biomedical Innovation, Osaka, Japan, [2]Instruments company, NIKON CORPORATION, Yokohama, Japan

Background: Cell density is a critical factor for control both growth and differentiation of human pluripotent stem (PS) cells including embryonic stem (ES) cells and induced pluripotent stem (iPS) cells. Despite the fact of the evolution of human PS cell culture techniques, counting cell numbers is still problematic. Therefore, we have developed a noninvasive cell counting method for human PS cells through analyzing live cell images.
Methods: H9 (WA09) human ES cells were cultured and then stained with a cell-permeant SYTO24 green fluorescent nucleic acid stain (Invitrogen). Phase contrast and fluorescent images of H9 were obtained during culture in a cell incubator observation system, BioStation CT (Nikon Corporation) and analyzed by a software CL-Quant (Nikon Corporation). Area of H9 colony coverage was measured from phase contrast images. Number of stained nuclei was counted from green fluorescent images. Immediately after imaging, the conventional cell counting by hemocytometer was performed for comparison.
Results: The nucleus counting and the conventional cell counting showed similar results. In the case total numbers of cells were above 1×10^4, cell numbers by nucleus counting were similar and reproducible with those by conventional cell counting. There was a significant correlation between the colony coverage area and the nucleus/cell counting. These results show that numbers of human PS cells can be estimated from the total colony coverage area through phase contrast imaging.

Conclusions: Thus we have developed a new noninvasive cell counting method. Furthermore, obtaining time-lapse phase contrast images enables us to monitor colony morphological changes and to calculate growth rate during human PS cell culture. Our noninvasive technique is useful for consistent seeding of human PS cells and high-throughput screening analysis of cell numbers, proliferation, and growth curves.

W-2092

VIRTUAL KARYOTYPING OF STEM CELLS BASED ON THEIR GLOBAL GENE EXPRESSION PROFILES

Uri Ben-David[1], Yoav Mayshar[2], Nissim Benvenisty[1]
[1]Hebrew Univ Stem Cell Unit, Jerusalem, Israel, [2]Dept. of Genetics, Harvard Medical School, Boston, MA, United States

The genomic instability of stem cells in culture, caused by their routine in-vitro propagation or by their genetic manipulation, is deleterious both for their clinical application and for their use in basic research. Frequent evaluation of the genomic integrity of stem cells is thus required, and is usually performed using cytogenetic or DNA-based methods, at variable sensitivities, resolutions and costs. These methods require access to the cells - or at least to DNA from the cells - and their application is usually limited to cell lines one has readily at hand. In contrast, gene expression microarrays have become a common tool in stem cell research and microarray data are frequently deposited in public databases.
Here we present a method that enables the accurate evaluation of the genomic integrity of stem cells based on their gene expression data. Once gene expression microarrays are generated from the cell lines of interest, they are compared to a database of similar gene expression profiles using two complementary bioinformatic analyses. The results obtained from these analyses are then combined to faithfully determine what genomic aberrations are present in the examined samples. This expression-based karyotyping method (which we termed "e-karyotyping") was initially validated by analyzing human embryonic stem cells (hESCs) with known chromosomal aberrations. We then e-karyotyped a large data set of human ESCs and conducted the first large-scale analysis of genomic integrity in human induced pluripotent stem cells (hiPSCs). Recently, we also applied e-karyotyping to mouse and rhesus PSCs, demonstrating that it is not restricted to human cells. Lastly, we also performed e-karyotyping to examine the genetic stability of human adult stem cells, namely hematopoietic stem/progenitor cells (HSPCs), neural stem cells (NSCs) and mesenchymal stem cells (MSCs), demonstrating that each stem cell type is prone to acquire a unique set of chromosomal aberrations, and that these aberrations are similar to those observed in tumors of the same cell lineage.
The current work provides a detailed protocol for using global gene expression profiles to determine the genomic integrity of stem cells, with an emphasis on PSCs. This e-karyotyping protocol describes how to properly organize gene expression microarray data, how to subject it to two complementary bioinformatic analyses, and how to interpret the results in a conservative manner, in order to generate an accurate estimation of the chromosomal aberrations in the autosomal genome of examined stem cell lines. This is an easy-to-follow protocol that every standard laboratory in the field will be able to adopt and implement.
*
This study is currently being revised for publication in Nature Protocols, and it follows our recently published works:
Mayshar*, Ben-David* et al, Cell Stem Cell, 2010
Ben-David et al, Cell Stem Cell, 2011
Ben-David and Benvenisty, Stem Cells, 2012

W-2093

FUNCTIONAL HETEROGENEITY OF UMBILICAL CORD BLOOD CD34 POSITIVE CELLS

Elena Afrimzon[1], Naomi Zurgil[1], Yana Shafran[1], Maria Sobolev[1], Ronit Goldman-Levi[2], Eilat Shinar[3], Mordechai Deutsch[1]

[1]Physics, Bar-Ilan University, Ramat Gan, Israel, [A]Public Cord Blood Bank Lab, [2]Magen David Adom, Ramat Gan, Israel

Background. The great heterogeneity in living cells provides the stable spatiotemporal function of the multicellular organism. UCB contains hematopoietic stem cell pools belonging simultaneously to both adult/mother and embryo/infantorganisms. Study of morphological and functional heterogeneity in the CD34 positive UCB cells at the single cell level may provide sufficiently detailed information which can contribute to the understanding of the biological activity of these cells and their potential use.

Methods. In this study, we present an imaging approach based on the cell retainer methodology for real-time investigation of the functional intracellular characteristics of UCB cells. This methodology enables repetitive, high-content correlative multi-parametric measurement and image analysis of individual cells within a population, during various manipulations, e.g. drug introduction, staining procedures, etc. [Deutsch M. et al, 2006]. Fresh UCB units from healthy newborns were obtained

from Magen David Adom Public Cord Blood Bank (Israel). Initially, red blood cells were sedimented using dextran and thereafter mononuclear cells were isolated using Ficoll-Hypaque. The separated mononuclear cells were loaded into the *LiveCell™ Array* and a panel of vital tests was performed at the level of individual cells. For instance, cell death rate (PI staining), cytoplasm membrane integrity and intracellular metabolism (the rate of fluorescein-diacetate hydrolysis by non-specific esterases calculated from repeated periodic measurements for each single cell), mitochondrial membrane potential (TMRM staining) and Heochst dye (HD) efflux. UCB mononuclear cells were sorted based on their CD34 fluorescence intensity (FI). The functional features of CD34 positive

versus negative cells, as well as the difference between subfractions of $CD34^{highFI}$ and $CD34^{low\ FI}$ cells were analyzed.

Results and Conclusions. On average, 8.8±3.7% of the total mononuclear cells exhibited positive staining with anti-CD34 antibodies. Living CD34 positive cells demonstrated significantly lower (p<0.001) MMP (TMRM dye FI) and significantly higher (p<0.02) HD FI in comparison to CD34 negative cells. In contrast, the intracellular non-specific esterases demonstrated a similar rate of FDA hydrolysis in CD34 positive and negative cells. Among CD34 positive cells ~30% exhibited high FI signal, while the rest had a low FI signal. The $CD34^{high\ FI}$ fraction did not efflux HD effectively, in contrast to $CD34^{low\ FI}$ cells which are associated with significantly lower staining (p<0.03). On the other hand, these two fractions of CD34 positive cells do not differ significantly with respect to MMP and ability to hydrolyze FDA.

Thus, freshly isolated CD34 positive mononuclear cells derived from UCB reveal a certain level of heterogeneity with respect to intracellular functional characteristics. The results obtained may be important for further basic investigations, and to applied research as well.

W-2094

BENEFICIAL EFFECT OF PAN-CASPASE INHIBITOR FOR CRYOPRESERVATION OF HUMAN UMBILICAL CORD DERIVED MESENCHYMAL STEM CELLS (HUC-MSCS)

Back Soo Shin, Su-Youne Han, Hyun Woo Lee, Jin Tac Kim, Hyeon Kyeong Lee, Seung Hoon Pak, Ji Hyang Kim, Byung-Rok Do

Biotechnology Research Institute, Hurim BioCell Inc., Seoul, Korea, Republic of

The cryopreservation of hUC-MSCs has become an important process for the therapeutic protocol, which includes stem cell transplantation. In the conventional cryopreservation protocol, using the high concentration of dimethylsulfoxide (DMSO) presented various cytotoxic effects for stem cell. Therefore, to reduce the cytotoxicity of DMSO, we studied the roles of non-permeable cryoprotective agents (Np-CPAs; pan-caspase inhibitor, antioxidant and disaccharide) for effective cryopreservation.

hUC-MSCs were cryopreserved with Group 1) 10% DMSO and 20% serum substitute supplement (SSS) as a control, Group 2) 5% DMSO and 5% SSS, Group 3) 5% DMSO and 5% SSS treated with 0.1M sucrose, 0.1M trehalose, 1mM antioxidant (1mM Glutamine) and 5% pan-caspase inhibitor (5% N-benzyloxycabonyl-Val-Ala-Asp-fluoro-

methylketone; zVAD-fmk), respectively. The relative survival rates of frozen-thawed cells were measured by trypan blue staining after 2 weeks of storage in a LN_2 tank. The population growth rate and doubling time of cultured cells were assessed using the MesenPRO medium for 5 weeks.
The relative survival rate was 76.74% (group1) of conventional cryopreservation protocol. And it was 70.38% (group2) of 5% DMSO, showed reduced relative survival rate than group1. Additional effect of 0.1M sucrose, 0.1M trehalose and 1mM glutamine (group3), showed similar relative survival rate compared with the control (group1), but it was increased relative survival rate than group2. The treatment with 5% zVAD-fmk (group3), showed a significantly higher relative survival rate (91.65%) than without it (group1 and group2) (p;0.027, p;0.026). The population growth rate and doubling time of cultured cells were showing similar results between w and w/o Np-CPAs.
These results were presented that the reduced concentration of DMSO by supplement of Np-CPAs, especially caspase inhibitor has an advantage to overcome the many side effects of cryopreservation in cell therapy.

iPS Cells

W-2102

GENOME EDITING OF ALPHA- SYNUCLEIN IN IPSCS FROM A PATIENT WITH MULTIPLE SYSTEM ATROPHY

Kun Bi[1], David Thompson[1], Spencer Hermanson[1], Kurt Vogel[1], J. William Langston[2], Birgitt Schuele[2]
[1]Primary and Stem Cell Systems, Life Technologies, Madison, WI, United States, [2]The Parkinson's Institute, Sunnyvale, CA, United States

Multiple system atrophy (MSA) is a neurodegenerative disorder of primarily glial origin. Clinically it is distinct from Parkinson's disease with predominantly autonomic failure and motor impairment. The motor symptoms generally respond poorly to dopaminergic therapy. Pathologically, the principal cellular targets are oligodendrocytes that show abundant glial cytoplasmic inclusion bodies consisting of alpha-synuclein aggregates and nigrostriatal degeneration. Protein misfolding and aggregation of alpha-synuclein is a common feature across synucleinopathies and an attractive target for drug development.
In order to study the contribution of α-synuclein to the disease phenotype of MSA, we generated induced pluripotent stem cells (iPSCs) from a patient with MSA and then created isogenic lines with SNCA deletional mutations using the transcription activation-like effector nucleases (TALEN) technology. We designed two sets of TALENs to create deletions in exon 2 of the SNCA gene in patient derived iPSCs via non-homologous end-joining (NHEJ). After functional verification of the SNCA TALENs carried out in HEK cells and confirmed with the Surveyor assay and sequencing, iPSCs from the MSA patient were edited with SNCA TALENs. Colonies that showed positive cuts in the Surveyor assay were picked and expanded. These colonies were further characterized by sequencing on the Ion Torrent PGM instrument to identify clonal populations with SNCA deletional mutations. We then differentiated both the edited lines and isogenic control iPSC line into neural stem cells and glial cells for functional characterization. In conclusion, TALEN technology can be applied to generate isogenic disease iPSCs for studying the contribution of SNCA in the disease phenotype of MSA.

W-2102

CHARACTERIZATION OF IPSC-DERIVED NEURONS CONTAINING A FAMILIAL ALZHEIMER'S DISEASE MUTATION

Shahad Rahawi[1], Grace Woodruff[2], Lawrence S. B. Goldstein[2]
[1]San Diego State University, San Diego, CA, United States, [2]University of California, San Diego, La Jolla, CA, United States

Alzheimer's disease (AD) is the leading cause of dementia worldwide, and AD cases are estimated to increase three-fold by 2050 due to an aging population. The pathological hallmarks seen in AD brains include amyloid-β (Aβ) plaques, neurofibrillary tangles of hyper-phosphorylated tau protein, synaptic loss, and neuronal death. Sequential proteolysis of amyloid precursor protein (APP) by beta and gamma secretases generates Aβ fragments, which are

the main constituents of the senile plaques. The majority of AD cases are sporadic, without a known genetic or environmental cause. However, there is a small subset of patients with dominantly inherited forms of the disease caused by mutations in APP and presenilin 1 and 2. IPSC technology allows us to capture the genomes of individuals with familial AD mutations and to differentiate iPSCs to neurons and glia to study the mechanisms that cause AD. Here we report the characterization of iPSC-derived neurons from cell lines containing the familial AD mutation, APP V717F. We report that the APP V717F mutation increases the Aβ42/40 ratio in purified neurons and increases APP β c-terminal fragments (βCTFs). Studying how an increased Aβ42/40 ratio and increased APP βCTFs contribute to AD will be vital for future therapeutic intervention.

W-2103

TALEN-MEDIATED INTRODUCTION OF APP SWEDISH AND MV POINT MUTATIONS IN AN IPSC MODEL OF FAMILIAL ALZHEIMER'S DISEASE

Nina D. Kloss, Sol M. Reyna, Lawrence S. Goldstein
Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA, United States

Alzheimer's Disease (AD) is a progressive and to-date irreversible neurodegenerative disease that presently affects more than 26 million people worldwide. This number is predicted to greatly increase by the year 2050. While more than 95% of AD cases occur sporadically without a known genetic cause, mutations in the amyloid precursor protein (APP) or presenilin genes (PS1 and PS2) have been attributed to rare autosomal dominant forms of familial AD (FAD). In this context, the Swedish mutation was identified as a genetic variation leading to FAD. This mutation alters the processing of APP by enhancing beta-secretase cleavage which leads to increased generation of both APP C-terminal fragments and pathogenic amyloid-β (Aβ) peptide. In contrast, the MV mutation has a single point mutation at the same site to inhibit beta-secretase cleavage. We are generating transcription activator-like effector nucleases (TALENs) to introduce these Swedish and MV point mutations in Craig Venter's cell line. TAL effectors are proteins secreted by plant pathogenic bacteria and bind to DNA in a modular fashion allowing for precise DNA targeting. The generation of isogenic iPSCs containing Swedish and MV mutations will provide the tools necessary to address the contribution of different APP fragments (namely APP CTFs and Aβ peptides) in driving axonal transport defects of organelles and vesicles, such as lysosomes and mitochondria.

W-2104

ABETA 42 TO 40 RATIOS IN HUMAN NEURONAL CULTURES ARE RESISTANT TO PHARMACOLOGICALLY RELEVANT CONCENTRATIONS OF GAMMA SECRETASE MODULATORS

Philipp Koch[1], Jerome Mertens[1], Kathrin Stüber[1], Patrick Wunderlich[2], Julia Ladewig[1], Rik Vandenberghe[3], Mathieu Vandenbulcke[4], Philip van Damme[3], Jochen Walter[2], Oliver Brüstle[1]
[A]Reconstructive Neurobiology, [B]Neurology, [1]University of Bonn, Bonn, Germany, [A]Neurology, [B]Psychiatry, [2]University Hospitals Leuven, Leuven, Belgium

Altered production, accumulation and aggregation of amyloid beta peptides (Aβ) in the brain are hallmarks of sporadic and familial Alzheimer's disease (AD). As increasing evidence suggests that elevated fractions of the highly amyloidogenic Aβ42 variant are causative in AD pathogenesis, γ-secretase modulators (GSM) that selectively lower Aβ42 emerged as potential therapeutic agents. Surprisingly and despite promising preclinical data, the GSMs so far tested in human clinical trials proved inefficient in delaying disease progression. While the reasons for these failures remain obscure, it is striking that most data on the potency of GSMs have not been generated in bona fide human neurons. The advent of human pluripotent cells (hPSC) has enabled the generation of human neurons in large quantities. Here we show that neurons derived from human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC) endogenously generate Aβ and that familial AD patient-specific iPSC-derived neurons exhibit an elevated Aβ42/40 ratio. However, exposure to GSMs at concentrations that can be clinically achieved in the brain and that were efficient in non-human cell models did not alter the ratio of Aβ42/40 in these neurons. Thus, our

data reveal an unexpectedly low responsiveness of authentic human neurons to pharmacological γ-secretase modulation, which might explain the observed clinical failure of GSMs.

W-2105

IN VITRO MODELING OF ALZHEMER'S DISEASE

Maya Mitalipova[1], Julien Muffat[1], Yun Li[1], Tenzin Lungjangwa[1], Ping Xu[1], Robert Moir[2], Doo Yeon Kim[2], Rudolf Tanzi[2], Rudolf Jaenisch[1]

[1]Whitehead Institute for Biomedical Research MIT, Cambridge, MA, United States, [2]Genetic and Aging Research Unit, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, United States

Alzheimer's disease (AD) is characterized by progressive dementia and brain deposits of the amyloid β protein (Aβ) as senile plaques and the microtubule-associated protein, Tau, as neurofibrillary tangles (NFT). Recent developments in induced pluripotent stem cells (iPSCs) derivation have allowed investigation of phenotypes of neurological diseases in vitro, and provide opportunities to generate human cell-based models that will be crucial for drug discovery, as well as to investigate disease mechanism.
We have generated iPS cells from patients with familial AD (FAD type 3), from a Canadian family with A246E mutation in the presenilin 1 (PS1) protein, and from non-demented kins. We transduced fibroblasts with doxycycline (dox) inducible, single Cre-excisable lentiviral vector encoding four reprogramming factors (Oct4, Sox2, Klf4 and c-Myc) flanked by lox-P sites as a polycistronic cassette. These cells also were transduced with a constitutive Cre-excisable lentiviral vector expressing M2rtTA. Patient and control iPS cells were, using SMAD signaling inhibition (dorsomorphin), differentiated to highly expandable neural progenitors and matured into the neuronal and glial phenotypes relevant to AD. This induced rapid and complete neural conversion of iPS cells under adherent culture conditions. In particular, we have generated glutamatergic excitatory neurons and astrocytes from each patient. To evaluate their usefulness as an AD model we measured the levels of Abeta-40 and Abeta-42 secreted from 8-10 weeks old neurons by sandwich ELISA. We show that total Aβ secretion is much higher in neuroglial cultures, making these a far better model to study the disease than fibroblasts. Then we compared the ratio of Abeta-42 to Abeta-40 to total Abeta secreted by control and mutant neurons. Mutant neurons showed higher ratio of Abeta-42/40 compare to control neurons. To determine whether soluble or non-soluble Abeta species are neurotoxic in vitro, and assess whether sub-types of neurons are selectively vulnerable we exposed control cells to varying concentrations of stressors, including pre-aggregated Aβ42 and soluble Aβ extract from supernatants from A246E human neurons. Toxicity assessment has been performed in parallel, using a vital dye (PrestoBlue), as well as endpoint immunohistological staining of neuronal dendrites (MAP2), astrocytes (GFAP) and mature synapses (Synapsin puncta). We have found that mutant neurons have less complex dendritic arbors and are more vulnerable to Abeta-42 s compare to control neurons.
In conclusion we describe phenotypic changes of human AD neurons in vitro, which will allow us to investigate the mechanism of the disease and effect of small molecules to rescue the AD phenotype.

W-2106

USING HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED MOTOR NEURONS TO ELUCIDATE THE ROLE OF MUTANT SOD1 IN MITOCHONDRIAL DYSFUNCTION AND TRANSPORT IN AMYOTROPHIC LATERAL SCLEROSIS

Theodore Peng[1], Evangelos Kiskinis[2], Steve Han[3], Kevin Eggan[1]

[A]Human Developmental and Regenerative Biology, [B]Molecular and Cellular Biology, [1]Harvard University, Cambridge, MA, United States, [2]Department of Neurology, Massachusetts General Hospital, Boston, MA, United States

Induced pluripotent stem cells (iPSCs) offer a new and exciting approach to disease modeling. Given the fact that they are patient specific (i.e. with identical genomes to the patients from which they are derived), iPSCs possess the ability to accurately replicate the phenotype of certain diseases. Our lab has created various iPSC lines from patients with familial cases of Amyotrophic Lateral Sclerosis (ALS), and has differentiated these lines into lower motor

neurons, the cell type affected in ALS. Mutations in Cu/Zn superoxide dismutase 1 (SOD1) are some of the most common causes of familial ALS, and the resulting mutant SOD1 protein is often found localized to the surface of mitochondria in motor neurons. This phenotype is found in conjunction with alterations in morphology, localization, and transport of mitochondria along the axons of motor neurons in SOD1 mutant mouse models. Furthermore, there have been reports of perturbations in calcium homeostasis and apoptosis activation of mitochondria. However, these observations have been made using non-human or non-motor neuron systems that overexpress mutant SOD1 protein, or human post mortem biopsies that offer only end-stage time points of the disease. In order to address these issues, we have used our iPSC-derived motor neuron in vitro model to elucidate the mechanisms and pathways underlying mitochondrial dysfunction, and to understand the influence mitochondria have on the ALS phenotype.

W-2107

A NOVEL CELL MODEL SYSTEM FOR STUDYING MECHANISMS OF SOD1-ASSOCIATED AMYOTROPHIC LATERAL SCLEROSIS.

Ilya Chestkov[1], Ekaterina Vasilieva[1], Ekaterina Khomyakova[1], Sergey Illarioshkin[2], Maria Lagarkova[1], Sergey Kiselev[1]

[1]Department of Epigenetics, Vavilov Institute of General Genetics, Russian Academy of Science, Moscow, Russian Federation, [2]Research Center of Neurology, Russian Academy of Medical Science, Moscow, Russian Federation

Amyotrophic lateral sclerosis (ALS) is a fatal disorder of motor neuron degeneration with unclear mechanisms and no effective treatment to date. The most commonly occurring mutations in patients with familial ALS (FALS) are found in the Cu, Zn superoxide dismutase (SOD1) gene and account for approximately 20% of all FALS. It is assumed that misfolded mutant SOD1 protein can be selectively toxic to motor neurons and therefore most cases of classic ALS can be considered as a conformational disorder. One of the most promising approaches to understand the primary causes of the disease is to generate a cell model system based on patient-specific induced pluripotent stem cells (iPSC). We have obtained and characterized human motor neurons with three different SOD1 mutations through differentiation of iPSCs derived from patients affected by SOD1-associated ALS. These motor neurons could be specifically visualized during differentiation process and sorted due to Hb9::eGFP-IRES-Puro motor neuron-specific reporter cassette introduced in each iPSC line. The resulting model system will allow using motor neurons in the co-cultivation experiments with other cell types (non-cell autonomous effect), and also test whether neuron death in ALS can be a cell autonomous process as well.

W-2108

ALS PATIENTS' MOTOR NEURONS EXHIBIT PREFERENTIAL IMPAIRMENT IN NEUROFILAMENT AND MITOCHONDRIAL DYNAMICS IN ABSENCE OF GLIA

Hong Chen, Kun Qian, Zhongwei Du, Jinagyuan Cao, Lisle W Blackbourn IV, Anthony Errigo, Cindy Huang, Melvin Ayala, Ling Yao, Su-chun Zhang
Waisman center, Madison, WI, United States

Amyotrophic lateral sclerosis (ALS) is characterized by presence of neurofilament tangles in cell bodies and axons followed by degeneration of motor neurons. The cause and underlying mechanisms of neurofilament aggregation and preferential motor neuron vulnerability remain unknown. Mutations in the Cu/Zn Superoxide Dismutase (SOD1) gene are associated with 20% familial forms of amyotrophic lateral sclerosis (ALS) and transgenic expression of multiple copies of the mutant forms of human SOD1 in animals indeed leads to neuronal degeneration. To gain insights into the process of motor neuron degeneration in ALS patients and to build a dynamic model for dissecting disease pathogenesis and for drug discovery, we established induced pluripotent cells (iPSCs) from patients with SOD1 mutations. Analysis of the iPSC-derived neurons indicated that the ALS hallmark pathology, neurofilament aggregation, appeared progressively in spinal motor neurons but rarely in non-motor neurons before glial cells have

developed in the system. The neurofilament aggregates were associated with deficits in mitochondrial function as well as transportation along axons. Expression of a single copy of the same mutant SOD1 in human embryonic stem cells (ESCs) by TALEN-mediated transgenesis resulted in the same pathological changes in motor neurons but not non-motor neurons that are differentiated from the transgenic hESCs. Genetic correction of the SOD1 mutation in the patient's iPSCs by TALEN-based homogeneous recombination prevented the above pathological phenotypes. These results indicate that the SOD1 mutations lead to structural and functional degeneration, preferentially in motor neurons and that the degenerative process occurs in a cell-autonomous manner. They also suggest the usefulness of the iPSC strategy for modeling the pathological process of ALS. Studies are under way to dissect out early events that lead to neurofilament and mitochondrial changes, to understand the preferential vulnerability of motor neurons, and to build platforms for drug discovery.

W-2111

APPLYING IPSC MODEL TO STUDY P53 MUTATION ASSOCIATED CANCER DISSEASE

Jie Su[1], Dung-Fang Lee[1], Betty Chang[1], Julian Gingold[1], Henia Darr[1], Carlos-Filipe Pereira[1], Avinash Waghray[1], Ran Brosh[1], Christoph Schaniel[1], Razmik Mirzayans[2], Ihor R. Lemischka[1]

[1]Department of Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY, United States, [2]Department of Oncology, University of Alberta, Edmonton, AB, Canada

Embryonic stem cells (ESCs) hold enormous promise for biomedicine. In vitro modeling of human disease has recently become possible due to induced pluripotent stem cell (iPSC) methodologies. Characterized by their ability to self-renew indefinitely and differentiate into all cell lineages of an organism like ESCs, iPSCs provide a powerful and unlimited source of cells to generate differentiated cells that can be used to elucidate disease pathogenesis, for drug discovery and development, toxicology screening, personalized healthcare and eventually cell transplantation-based therapies. Here, we apply iPSC technology to investigate p53 mutation-associated cancer disease. This will create a "disease in a dish" platform to elucidate the underlying pathogenesis caused by these p53 mutations.
We obtained fibroblasts from a family with heterozygous Gly245Asp (G245D) mutation in the TP53 gene. We have generated integration-free iPS cell lines from three patients and two unaffected family members by Sendai virus (SeV)-based delivery of the 4 Yamanaka reprogramming factors, OCT4, SOX2, KLF4 and c-MYC. These iPS clones express hESC pluripotency factors NANOG, OCT4 and SOX2, hESC surface markers (TRA-1-81 and SSEA4), and alkaline phosphatase (AP). iPS lines were tested for loss of Sendai virus by PCR. The G245D mutations in SeV-free iPSC lines have been confirmed in 3 patients and wild-type status confirmed in 2 related family members. The iPSC lines were further characterized to verify normal karyotype, expression of NANOG, OCT4 and SOX2 pluripotency markers at levels comparable to hESCs and reduced OCT4 promoter methylation compared with original fibroblasts. In order to obtain a comprehensive picture, we will further identify genome-wide differences between WT and mutant p53 (G245D) lines by transcriptome, miRNAs, interactome and ChIP-seq approaches. Integrating these data will provide insights into the tumor suppressor role of p53. Collectively, applying iPSC technology to study cancer disease will provide an invaluable avenue to unravel the tumor-promoting role of p53 mutants.

W-2112

MODELING IDIOPATHIC AUTISM USING INDUCED PLURIPOTENT STEM CELLS

Maria Carolina Marchetto[1], Patricia Beltrão-Braga[2], Haim Belinson[3], Yanelli Nunez[1], Karen Pierce[4], Eric Courchesne[4], Antony Wynshaw-Boris[3], Alysson Muotri[5], Fred Gage[1]

[1]Salk Institute, La Jolla, CA, United States, [2]University of Sao Paulo, Sao Paulo, Brazil, [3]University of California San Francisco, San Francisco, CA, United States, [A]Autism Center of Excellence, [4]University of California San Diego, La Jolla, CA, United States

Autism spectrum disorders (ASD) are complex neurodevelopmental diseases that affect about 1% of children in the United States. Such disorders are characterized by deficits in verbal communication, impaired social interaction, as

well as limited and repetitive interests and behavior. The precise mechanisms that cause autism, however, remain unknown. Recently, neuropathological imaging and genetic studies have provided important insights into ASD, and these studies have led to two major hypotheses for autism pathogenesis: altered brain growth and dysfunctional neuronal networks (Courchesne et al. 2003; Dementieva et al. 2005; Garber 2007). The major impediment to testing these and other hypotheses of autism is the lack of relevant animal and cell models. The direct study of live brain tissue from ASD patients is unfeasible, and no suitable animal models can adequately reproduce the complicated structure and function of the human brain to study social behavior and language acquisition. Recently, reprogramming of human somatic cells to a pluripotent state by over-expression of specific genes into induced pluripotent stem cells, or iPSCs (Takahashi et al., 2007) has provided an exciting opportunity to produce a relevant human cellular model of human complex neurogenetic diseases, and iPSCs have been generated for several neurological disorders and diseases, including Rett syndrome, a syndromic ASD (Marchetto et al. 2010). Here we show the generation of 8 idiopathic autistic iPSC lines and 5 age/gender-matched control lines. We also performed differentiation into neural progenitor cells (NPC) and mature neurons in culture. We are now revealing cellular phenotypes that can directly test the two current models for autism pathogenesis abovementioned (cell proliferation, cell migration and proper neuronal maturation). Knowledge of whether altered brain growth and/or dysfunctional neuronal networks play a role in the development of autism will inform studies in human patients. These studies should provide important and novel insights into the development of ASD and will test whether cellular markers are present in patient-derived NPCs and neurons from iPSCs. Knowledge of the biological causes of neural maldevelopment in ASD would likely lead to the development of clinically useful biomarkers of risk for this disorder in young, pre-symptomatic infants, which may lead to the development of novel therapies.

W-2113

IN VITRO MODELING OF NIEMANN-PICK TYPE C DISEASE USING PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

Dorothea Maetzel[1], Haoyi Wang[1], Sovan Sarker[1], Lina Abi Mosleh[2], Qing Gao[1], Maisam Mitalipova[1], Rudolf Jaenisch[1]

[1]Whitehead Inst for Biomedical Research, MIT, Cambridge, MA, United States, [2]Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, TX, United States

Patient-specific induced pluripotent stem (iPS) cells derived from somatic tissues provide a unique tool to study human diseases *in vitro*. The potential of iPS cells to generate unlimited quantities of disease affected cell types make them a realistic source for the analysis of disease underlying mechanisms, small molecule drug screening and autologous transplantation. Therefore patient specific iPS cells are potentially useful to develop new strategies to treat patients suffering from various degenerative diseases.
Niemann-Pick disease type C (NPC1) is an inherited, progressive neurodegenerative lipid storage disorder caused by mutations in the NPC1 or NPC2 gene. Mutations in these genes result in an accumulation of unesterified cholesterol in the late endosomes / lysosomes (LE/L) and an impairment of the cholesterol export from the LE/L to the endoplasmic reticulum (ER). The first clinical symptoms are often hepatosplenomegaly and cholestasis. The course of the disease is dramatic and subsequently leads to the death of the patient.
In the presented study we have generated vector-free iPS cells from patients suffering of NPC1 disease. Using different biochemical approaches we were able to evaluate and identify disease-related phenotypes in different cell types including cells of the neuronal and hepatic lineage. We have, using transcription activator-like effector nucleases (TALENs) mediated gene editing, generated isogenic NPC1 and control iPS cell lines and are investigating the effect of NPC1 deficiency on disease relevant phenotypes in neuronal and hepatocyte-like cells.

W-2114

MODELING DISEASE SEVERITY IN AN IPSC-BASED IN VITRO MODEL OF FAMILIAL DYSAUTONOMIA

Nadja Zeltner[1], Gabsang Lee[2], Faranak Fatahi[3], Fabien Lafaille[4], Jason Tchieu[1], Lorenz Studer[1]

[1]Developmental Biology/Center for Stem Cell Biology, Memorial Sloan-Kettering Cancer Center, New York, NY, United States, [2]Institute for Cell Engineering, John's Hopkins University, Baltimore, MD, United States, [3]Graduate School, Cornell University, New York, NY, United States, [4]St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller University, New York, NY, United States

Pluripotent stem cells have the potential to become any cell of the body and a key goal in disease modeling studies is to utilize cells of interest to understand the pathology associated with the disease. Familial Dysautonomia (FD) is a neurodegenerative/neurodevelopmental genetic disease that we employ to establish the principle of in vitro disease modeling as a valid alternative/addition to animal models of human diseases. Thus, offering the possibility to study disease specifics, accomplish drug screening and drug testing. Using FD-patient derived cells, we have previously shown that FD can be successfully modeled by using the induced pluripotent stem cell (iPSC) technology. iPSCs were generated from FD patients and differentiated into neural crest precursors (the cells specifically affected in FD). Three disease characteristics of FD were reproduced in vitro in neural crest precursors indicating that FD can be modeled effectively. However, FD patients display a range of symptoms, from severe and mild (both homozygous) to the asymptomatic, heterozygous carrier phenotype. We aim to investigate if this range of severity can be reproduced using our iPSC-based disease model and determine the mechanism behind differences in disease severity.
Using iPSCs from FD patients with severe, mild, absent (heterozygous) and healthy control patients, we differentiated them into neural crest progenitor cells and examined their in vitro phenotype. Aside from the FD-specific mis-splicing of IKBKAP, there is no difference between severe and mild patients in neural crest progenitors, suggesting no difference in the ability to generate the early neural crest lineage. However, the generation of Mash1-positive autonomic precursor cells is greatly affected in cells derived from severe patients, while cells from mild patients can generate equal numbers of autonomic precursor cells compared to controls. This suggests a specific defect in autonomic neurogenesis in severe but not mild patients, leading us to hypothesize that severe patients have both a neurodevelopmental and neurodegenerative defect, while mild patients may only have a neurodegenerative defect. To further test this hypothesis we are addressing the neurodegenerative aspect of the disease in more detail. To this end, we are employing our recently established differentiation protocols to generate peripheral sensory neurons, cells specifically affected by degeneration in FD patients. Survival capacities are being studied in these cells comparing sensory neurons derived from severe, mild or control FD patients.

W-2115

IDENTIFICATION OF NOVEL TARGETS USING PSEN1 FAMILIAL ALZHEIMER'S DISEASE HIPSC-DERIVED NEURAL PROGENITORS

Andrew A. Sproul[1], Samson Jacob[1], Deborah Pre[2], Soong Ho Kim[3], Michael W. Nestor[1], Matthew Zimmer[1], Soline Aubrey[2], David J. Kahler[1], John F. Crary[2], Ottavio Arancio[2], Sam Gandy[3], Scott A. Noggle[1]
[1]The New York Stem Cell Foundation, New York, NY, United States, [2]Pathology & Cell Biology and the Taub Institute for Research on Alzheimer's Disease, Columbia University, New York, NY, United States, [3]Neurology and Psychiatry and the Alzheimer's Disease Research Center, Icahn School of Medicine at Mount Sinai, New York, NY, United States

The most prevalent theory for the underlying cause of Alzheimer disease (AD) is the "amyloid hypothesis", in which toxic oligomerogenic forms of Abeta, a cleavage product of APP, accumulate and cause neuronal dysfunction and cell death. Presenilin 1 (PSEN1) encodes the catalytic subunit of γ-secretase, and PSEN1 mutations are the most common cause of early onset familial Alzheimer's disease (FAD). FAD mouse models and human AD pathology suggest potential deficits in adult neurogenesis and newborn neuron survival, indicating a potential important role for NPCs in the disease. In addition, the brains of early-onset Alzheimer's patients might have developmental alterations that could affect disease initiation. Thus, we interrogated potential difference in human FAD IPSC-derived NPCs, which have not been studied to date. We furthermore asked whether PSEN1 NPCs, a more homogenous population than the wide spectrum of mature neurons produced by a general neuronal differentiation, could be used to identify novel molecules potentially important for early events in AD. We generated induced pluripotent stem

cells (iPSCs) from affected and unaffected individuals from two families carrying PSEN1 mutations, the “Canadian” (FAD1, A246E PSEN1 mutation) and the “Italian” (FAD4, M146L PSEN1 mutation) FAD kindreds. iPSCs were characterized using a variety of quality-control assays, and eight iPSC lines were selected to serve as a core set for the majority of our experiments We successfully differentiated iPSCs into NPCs and electrophysiologically-active neurons, and PSEN1 NPCs have a small neurogenic advantage over control NPCs. PSEN1 mutant fibroblasts and NPCs produced greater ratios of Abeta42 to Abeta40 relative to their control counterparts, with the elevated ratio even more apparent in PSEN1 NPCs than in fibroblasts. Molecular profiling identified 14 genes differentially regulated in PSEN1 NPCs relative to control NPCs. We confirmed these hits in brains from common, sporadic AD cases with advanced or intermediate pathology by qPCR and by comparison to published transcriptomes of laser captured microdissected (LCM) cortical neurons from brains with AD pathology. Five of these targets showed differential expression in late onset AD/Intermediate AD pathology brains. Several of our gene hits have potential roles in human CNS development. Therefore, in our PSEN1 iPSC model, we have reconstituted an essential feature in the molecular pathogenesis of FAD, increased generation of Abeta42/Abeta40, and identified potential new therapeutic targets.

W-2116

INVESTIGATION OF PRION-LIKE PROPAGATION OF TAU USING NEURONS DERIVED FROM IPSCS

Patrick C. Reilly[1], Shauna Yuan[2]

[1]San Diego State University, San Diego, CA, United States, [2]Neurosciences, University California San Diego, La Jolla, CA, United States

Alzheimer’s disease is a neurodegenerative disorder affecting 5.4 million people nationwide and is the sixth leading cause of death in America. Lack of any treatment for this disease has caused death rates to rise while advancements in treatment for other leading causes of death in the United States have resulted in a statistically significant decrease in death rates. Progress in the treatment of Alzheimer’s disease has been inhibited by an insufficient understanding of disease pathology. Human and murine models for investigation exist, but Alzheimer’s disease is a human specific disease and acquiring neuronal cell lines from patients is difficult, expensive, and in short supply. Given the circumstances, there is a clear necessity for an easily accessible system which allows for the study of disease pathology and drug treatment in a human cell model. Our use of induced pluripotent stem cell (iPSC) derived neurons with mutations commonly found in individuals with Familial Alzheimer’s Disease (FAD) provides a unique, in vitro - human cell model for pathological investigation of Neurofibrillary Tangles (NFT), aggregates of misfolded tau protein, and Amyloid-β (Aβ) plaques, both of which play a significant pathological role in Alzheimer’s disease. Murine in vivo investigations of NFTs have demonstrated prion-like propagation of tau aggregates through anatomically connected nervous tissue. Consistent with disease progression, NFTs are associated with deafferentation and eventual neurodegeneration via propagation through neural networks. Recent work has shown both axonal and dendritic bulk-endocytotic uptake of aggregates, but the specific species, toxic processes, and mechanism of release into cellular cytosol and extracellular space remain largely unknown. Using iPSC derived neurons with FAD defects; we explore tau pathology by comparing diseased lines to wild type (WT) controls via transformed expression of a fluorescently labeled, tau aggregate promoting plasmid. Preliminary data confirms mutant tau plasmid induced aggregation in HEK 293T cells. Furthering this work through mutant tau plasmid transformation of iPSC derived neurons, we expect to see different cellular responses between WT and FAD cell lines to the tau aggregates. We anticipate the use of iPSC derived FAD and WT neurons as an in-vitro cell model will provide new insights in Alzheimer’s disease pathology and treatment.

W-2117

DISEASE-SPECIFIC INDUCED PLURIPOTENT STEM CELLS AS A USEFUL STEM CELL MODEL FOR HUNTINGTON’S DISEASE

Kyung-Ah Choi

Department of Biomedical Science, College of Health Science, Korea University, Seoul, Korea, Republic of

Huntington's disease (HD) is a neurodegenerative disorder caused by an expansion of CAG tri-nucleotide repeats that results in neuronal dysfunction and death. Neuronal death occurs in many brain regions, while degeneration of DARPP-32$^+$ GABAergic neurons in the striatum underlies motor dysfunction in HD. Recently, several groups have already reported the efficient differentiation of DARPP-32$^+$ GABAergic neurons from human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) of normal and HD patient fibroblasts. In this study, we showed that normal and HD iPSC lines were successfully established and characterized from normal and HD patient fibroblasts carrying an expanded CAG triplet-repeat tract, respectively. In our experiments to confirm pluripotency of normal and HD iPSCs using RT-PCR and immunocytochemistry, the expression of various pluripotent marker genes and proteins, such as OCT4, SOX2, C-myc, Klf4, Nanog, SSEA4, and TRA-1-60, was similar to those of hESCs. In our *in vitro* differentiation experiments, the normal and HD iPSC lines were differentiated into the DARPP-32$^+$ GABAergic neurons using MS5 stromal feeder cells (MS5) and culture media containing various cytokines and small molecules. The hiPSCs were differentiated into striatal GABAergic neurons, which were stained with various markers DARPP-32, GABA, GAD65/67, and βIII-tubulin. The normal hiPSCs were differentiated into approximately 87 % βIII-tubulin$^+$ (Tuj1$^+$) neurons out of total cells, and were 86% GABA$^+$ out of Tuj1$^+$ cells. The HD iPSCs were differentiated into approximately 85% Tuj1$^+$ neurons out of total cells, and were 81% GABA$^+$ out of Tuj1$^+$ cells. Interestingly, however, we found distinct expression patterns of various GABAergic markers between normal and disease hiPSCs in our RT-PCR experiments. Taken together, these results suggest that the pathogenesis of HD may be implicated to different expression of key genes, and that our HD iPSC lines could be provided as useful stem cell models to study the pathological mechanisms of HD and to screen the drug for HD disease treatments.

W-2118

HUMAN INDUCED PLURIPOTENT STEM CELLS FOR CELL REPLACEMENT THERAPY IN HUNTINGTON'S DISEASE.

Narawadee Choompoo, Ngoc-Nga Vinh, Claire Kelly, Anne E. Rosser
BRG, School of Biosciences, Cardiff University, Cardiff, United Kingdom

Huntington's disease (HD) is a neurodegenerative condition caused by a mutation in the huntingtin gene (HTT). The extended CAG repeat ultimately leads to loss of medium spiny neurons (MSNs) in the striatum of the HD brain. Cell replacement therapy using primary human fetal tissue has shown 'proof of principle' as a strategy to treat this genetically inherited disease[1]. However, alternative cell sources need to be identified to overcome the ethical and logistical issues that are associated with using human fetuses. Induced pluripotent stem (iPS) cells were first introduced by Yamanaka in 2006 by direct reprogramming of fibroblasts to ES-like cells by using four defined factors (Oct4, Sox2, Klf4, c-Myc)[2]. This landmark discovery paves the way to produce an unlimited cell source behaving similarly to an ES cell. Human iPS cells have certain advantages for clinical application in that they overcome some of the ethical issue associated with hES cell derivation, and may overcome immune rejection by opening the way for autologous transplantation. Here we attempt to generate iPS cells suitable for safe clinical use by introducing reprogramming factors using the *piggyBac* Transposon[3,4] transduction system to human fetal fibroblasts and fetal neural stem (FNS) cells. Fetal cells may be more readily reprogrammed to a pluripotent state and may retain "epigenetic memories" pertaining to their cells of origin, thus rendering them more ready to differentiate back to that cell subtype. The generation of cells that are differentiated to precisely the same subtype lost in a specific condition is critical for functional benefit following transplantation of those cells into the brain. In this study, we have derived iPS cell lines from human fetal whole ganglionic eminence (the putative striatum) and recently validated their pluripotentcy and differentiation potential. To date, we have determined the pluripotential nature of one iPS cell line using a range of measures, including a genetic profile. The established iPS cell line has properties similar to human embryonic stem (ES) cells in morphology, surface antigen, and proliferation. We are applying a strategy for removal of the transduction vector into the established primary iPS cell line to generate vector-free iPS cells to directly differentiate into neural progenitor cells and ultimately MSNs-like cells by using published protocols, and continue
to generate iPS cells from a variety of cell sources.

W-2121

JMJD3 NEGATIVELY REGULATES REPROGRAMMING THROUGH HISTONE DEMETHYLASE ACTIVITY DEPENDENT AND INDEPENDENT PATHWAYS

WEI ZHAO[1], Qingtian Li[1], Stephen Ayers[2], Yifeng Gu[1], Yidong Chen[3], Helen Yicheng Wang[1], Rongfu Wang[1]
[A]The Center for Inflammation and Epigenetics, [B]Genomic Medicine, [1]The Methodist Hospital Research Institute, Houston, TX, United States, [2]Department of Epidemiology and Biostatistics and Greehey Children's Cancer Research Institute, The University of Texas Health Science Center at San Antonio, San Antonio, TX, United States

Although somatic cell reprogramming to generate inducible pluripotent stem cells (iPSCs) is associated with profound epigenetic changes, the roles and mechanisms of epigenetic factors in this process remain poorly understood. Here we identify Jmjd3 as a potent negative regulator of reprogramming. Jmjd3-deficient MEFs produced significantly more iPSC colonies than did wild-type cells, while ectopic expression of Jmjd3 markedly inhibited reprogramming. We show that the inhibitory effects of Jmjd3 are produced through both histone demethylase-dependent and -independent pathways. The latter pathway is entirely novel and involves Jmjd3 targeting of PHF20 for ubiquitination and degradation via recruitment of an E3 ligase, Trim26. Importantly, PHF20-deficient MEFs could not be converted to fully reprogrammed iPSCs, even with knockdown of Jmjd3, Ink4a or p21, indicating that this protein exerts predominant effects on reprogramming. Our findings demonstrate a previously unrecognized role of Jmjd3 in cellular reprogramming and provide molecular insight into the mechanisms by which the Jmjd3-PHF20 axis controls this process.

W-2122

ELUCIDATING REPROGRAMMING OF HUMAN FIBROBLASTS TO INDUCED PLURIPOTENT STEM CELLS (iPSCs)

Eriona Hysolli[1], Yoshiaki Tanaka[1], Xinghua Pan[1], Kun-Yong Kim[1], Michael Snyder[2], Sherman Weissman[1], In-Hyun Park[1]
[1]Yale University, New Haven, CT, United States, [2]Stanford University, Stanford, CA, United States

Reprogramming generates induced pluripotent stem cells (iPSCs), which are similar to embryonic stem cells (ESCs) and possess the features of self-renewal and pluripotency. iPSCs offer great opportunities for the biomedical sciences. They can be used as sources of autologous cell therapy, in vitro cellular models, and drug screening. The successful use of iPSCs largely depends on the thorough understanding of the reprogramming process. Reprogramming human somatic cells requires a long time, and yields a very heterogeneous population of cells which may not be easily identified as bona-fide iPSCs. Thus, understanding how reprogramming occurs is critical to generate iPSCs safely applicable in therapy. We have reprogrammed human primary fibroblasts to iPSCs using four reprogramming factors (OCT4, SOX2, KLF4, and c-MYC). At weeks 1, 2, 3, and 4, we FACS sorted populations of cells using a combination of cell surface markers (CD13, SSEA4, TRA160), and silencing of GFP-carrying four factor retrovirus. We obtained around 1,000 - 20,000 cells for each sample, which were subsequently amplified and sequenced using RNA-Seq technology.

W-2123

OCT4 INDUCES PLURIPOTENCY THROUGH THE MODULATION OF ASH2L-MEDIATED HISTONE 3 LYSINE 4 METHYLATION

Bing Liao, Junjie Gu, Ying Jin
Shanghai Jiao Tong University School of Medicine, Shanghai, China

Reprogramming somatic cells
into induced pluripotent stem cells (iPSCs) using defined factors represents a
groundbreaking achievement for stem cell research and regenerative medicine. However,

the efficiency of iPSC generation, particularly when using genome insertion-free protocols, is very low, representing one of the major obstacles to the therapeutic application of reprogrammed cells. Elucidation of the mechanisms underlying induced pluripotency is important for the improvement of reprogramming efficiency. Experimental evidence indicates that expression of the 4 reprogramming factors (Oct4, Sox2, c-Myc and Klf4) at an optimal stoichiometry is critical for efficient reprogramming, and that the Oct4 dosage is a key parameter. Previous studies in our laboratory demonstrated that Wwp2, a HECT-type E3 ubiquitin ligase, specifically mediates Oct4 ubiquitination and represses transcriptional activity of Oct4. Furthermore, our results indicate that Wwp2-catalyzed ubiquitinated Oct4 proteins are subject to degradation by 26S proteasomes during the differentiation process of pluripotent stem cells. In this study, we identified 5 lysine residues on Oct4 as ubiquitin-conjugation sites using mass spectrometry. Mutation of the 5 lysine residues abrogated Wwp2-catalyzed Oct4 ubiquitin modification and enhanced the protein stability of Oct4 in differentiated cells. Based upon these results, we hypothesized that Oct4 defective in ubiquitin modification by Wwp2 might give rise to a higher reprogramming efficiency than wild type Oct4. To test the hypothesis, mutant Oct4 (Oct4-5R, the 5 lysine residues were mutated to 5 arginine) or wild-type Oct4 (Oct4-WT) was introduced into MEF (mouse embryonic fibroblasts) cells together with Sox2 and Klf4. Intriguingly, the Oct4-5R containing cocktail led to a reprogramming efficiency 10 times higher than that of the cocktail containing Oct4-WT. Consistently, Wwp2 null MEF cells had a significantly higher efficiency in pluripotency induction than wild type MEF cells, suggesting that Wwp2-mediated Oct4 ubiquitination represses pluripotency induction in MEF cells, probably through down-regulating the level of ectopically expressed Oct4 proteins. However, further investigation showed that the enhancement in the protein stability of the Oct4-5R mutant could not entirely account for its ability to improve the generation of iPSCs. Instead, we found that the higher pluripotency induction efficiency elicited by Oct4-5R could be attributed to the enhancement of Ash2l-mediated histone 3 lysine 4 methylation. Therefore, this study not only opens a new way to improve iPSC generation but also sheds light on the reprogramming mechanisms involving Oct4. Moreover, our results reveal how the reprogramming factor coordinates with the epigenetic regulator to control the induction of pluripotency.

W-2124

REACTIVATION OF X CHROMOSOME DURING HUMAN SOMATIC CELL REPROGRAMMING

Kun-yong Kim
Yale university, New haven, CT, United States

Induced pluripotent stem cells (iPSCs) are generated by reprogramming somatic cells with four transcription factors (Oct4, Sox2, Klf4, and c-Myc). Reprogramming causes fibroblasts to acquire an ESC-like epigenetic state, which includes expression of non-coding RNA, DNA and histone modification, and changes in X chromosome status. In recent studies, human female iPSCs with one active X chromosome or two active X chromosomes were found, suggesting that retention and reactivation of inactive fibroblast X chromosome could occur during reprogramming. X chromosome status in human female iPSCs in very important for future applications in in vitro and in vivo studies, but when and how the X chromosome reactivation (XCR) occurs during reprogramming is not yet fully understood. Here, we set out to elucidate the X chromosome reactivation during reprogramming of human female somatic cells. Interestingly, we found that there is a wave of transition in X chromosome status during and after reprogramming.

While cells actively undergoing reprogramming show the XCR, completion of reprogramming results in X chromosome inactivation, followed by XCR in select iPSC clones. Our findings show the dynamic X chromosome change occurs during human female somatic cell reprogramming.

W-2125

REPROGRAMMING TO PLURIPOTENCY BY SALL4, NANOG, ESRRB AND LIN28 PRODUCES HIGH QUALITY IPSCS WITH HIGH SUCCESS RATE AND DEFINES A MOLECULAR SIGNATURE OF IPSC DEVELOPMENTAL COMPETENCE

Yossi Buganim, Styliani Markoulaki, Batool Akhtar-Zaidi, Rudolf Jaenisch
WIBR, Whitehead Intitute, Cambridge, MA, United States

induced pluripotent stem cells (iPSCs) exhibit high variation in their developmental potential and a large proportion of them are of low developmental capacity as measured by stringent pluripotency assays such as chimera formation and tetraploid complementation assay (4n assay). As a result a large number of iPS colonies need to be screened in an attempt to find a high quality iPS line. This problem is even more amplified in human cells because they lack stringent pluripotency assays. Thus, finding a protocol that yields, with a high success rate, high quality iPSCs is an indispensable prerequisite for future iPSC-based therapy. Here we show that ectopic expression of Sall4, Nanog, Esrrb and Lin28 (SNEL) in mouse embryonic fibroblasts (MEFs), although produced low number of iPS colonies, generated with high efficiency high quality iPSC colonies as compared to Oct4, Sox2, Klf4 and c-Myc (OSKM). SNEL-iPSC colonies contributed to high-grade chimaeras and produced “all-iPS” mice by 4n assay. Whole genome transcriptional profile and bisulfite sequencing revealed distinct signatures of 1765 genes and 112 differentially methylated regions (4n-DMRs) that robustly distinguish lines that are 4n proficient from those that are not. Taken together, our results suggest that reprogramming factor combinations are critical determinants of iPSC quality and appropriate resetting to the pluripotent transcriptional and epigenetic states.

W-2126

PATIENT-DERIVED CONGENITAL ERYTHROPOIETIC (CEP) CELLS DISPLAY PHOTOSENSITIVITY DURING REPROGRAMMING

Suzanna Katz[1], Caroline Hendry[2], Dung-Fang Lee[2], Senkottuvelan Kadirvel[3], Sonia Clavero-Villarrub[3], David Bishop[3], Robert Desnick[3], Ihor Lemischka[2]
[A]Pharmacology and Systems Biology, [B]Developmental and Regenerative Biology, [C]Genetics and Genomic Sciences, [1]ICAHN School of Medicine at Mount Sinai, New York, NY, United States

Congenital Erythropoietic Porphyria (CEP) is a rare disorder of heme biosynthesis caused by an autosomal recessive mutation in the enzyme uroporphyrinogen III synthase (URO-synthase), which results in deficiency of heme production and the accumulation of porphyrins in the body. CEP is one of the most severe Porphyrias (a group of disorders affecting the heme biosynthetic pathway), with symptoms beginning soon after birth or in early childhood. Skin photosensitivity is one of the major clinical manifestation of porphyrin, resulting in severe blistering and scarring, often to the point of disfigurement. Patient samples of two unrelated patients with CEP were obtained in order to develop an in vitro disease model of CEP using stem cell technologies. Patients B1199 and B1340 have identical homozygous mutations that result in the most severe manifestation of CEP (resulting from <1% UROS activity). Thus, reprogramming fibroblasts from CEP patients into induced Pluripotent Stem Cells (iPSCs) would provide opportunities not only for greater understanding of disease pathobiology, but also for pharmacological testing and gene therapy clinical applications. However, since iPSCs are thought to be in an ‘open’ chromatin state, it was reasoned that promiscuous heme pathway activation would impede iPSC formation in CEP cells due to porphyrin induced photosensitivity. In order to determine if iPSC formation is light sensitive, CEP (B1199) and WT fibroblasts were infected with lentivirus and observed in light, dark, and UV conditions (daily exposure to 405nm light) during the reprogramming process. After several weeks, ~5-fold less Nanog+ colonies were observed in the light conditions, whereas the number of colonies from the WT cells remained the same. No colonies formed under the UV conditions, however

these conditions also drastically reduced the number of WT colonies too. Since photosensitivity was observed even during the reprogramming of CEP fibroblasts into the pluripotent state, iPSCs have been made via Sendai virus for the CEP Patients B1199 and B1340 in 100% dark conditions under far red light. This attempt yielded many colonies per line, several of which have been picked and expanded to passage 14. Established patient-derived iPSCs will allow us to elucidate the mechanism of light sensitivity in CEP and provide the ability to test clinical interventions in a safe and scalable way.

W-2127

USING HUMAN PATIENT SPECIFIC IPSC DERIVED EYE CUPS TO INTERROGATE AND TREAT INHERITED RETINAL DEGENERATION

Budd A. Tucker[1], Robert F. Mullins[1], Arlene Drack[1], Kristin Anfinson[1], Emily Kaalberg[1], Edwin M. Stone[2]
[1]Ophthalmology, University of Iowa, Iowa City, IA, United States, [2]Ophthalmology, University of Iowa, HHMI Investigator, Iowa City, IA, United States

Purpose: Retinitis pigmentosa (RP) is a genetically heterogeneous heritable disease characterized by apoptotic death of light sensing photoreceptor cells. Mutations in as many as 100 different genes are predicted to cause this disease. Many genes such as USH2A, are large and highly polymorphic and thus it is useful to be able to functionally confirm potential new disease-causing mutations in retinal cells prior to development of gene and/or cell based therapies. This is especially true for non-exomic mutations whose pathogenicity is difficult or even impossible to confirm at the DNA level. The purpose of this study was to develop patient specific retinal tissue for the interrogation of potential disease causing mutations and for development of new gene- and cell-based therapies.
Methods: iPSCs were generated via transduction of human keratinocytes, obtained from patients with USH2A-associated retinitis pigmentosa, using the transcription factors Oct4, Sox2, C-Myc and KLF4. iPSC potency was analyzed via ICC, WB, and teratoma formation. Capacity for retinal differentiation was determined via ICC, rt-PCR, and WB analysis of recoverin, rhodopsin, ROM-1, R/G opsin, B-opsin, and tubulin. To determine the capacity for cellular integration and photoreceptor maturation, subretinal transplantation into CRB1 null mice was performed. Pathogenicity of USH2A mutations was determined via rt-PCR targeted against USH2A.
Results: iPSCs generated from a patient with suspected USH2A associated RP were generated. Following differentiation, eyecups containing both RPE and neural retina were generated. Isolation, expansion and focused analysis of the pigmented and non-pigmented layers of patient specific eye cups allowed us to confirm RPE and photoreceptor cell lineage. Likewise transplantation of cells obtained from the neural retina into CRB1-null mice gave rise to newly generated photoreceptor cells. Analysis of the USH2A transcript within the neural retina revealed that a suspected splice site mutation within IVS40, induced exonification of the intron and insertion of a premature stop codon. In addition, a novel mutation identified via next generation whole exome sequencing was also confirmed within the iPSC derived neural retina of the patient.
Conclusions: By combining exome sequencing and induced pluripotent stem cell (iPSC) technologies we have been able to definitively demonstrate the pathogenicity of two disease-causing mutations in a patient with late onset non-syndromic USH2A associated RP. These findings have enabled us to proceed with patient specific studies focused on USH2A gene correction and photoreceptor cell replacement.

W-2128

STUDY OF MECP2 FUNCTION IN RETT SYNDROME-DERIVED INDUCED PLURIPOTENT STEM CELLS USING TALEN TECHNOLOGY

Ileana Slavin[1], Pablo Perez-Pinera[2], Charles A. Gersbach[2], Jeanne Loring[1]
[1]The Scripps Research Institute, La Jolla, CA, United States, [2]Duke University, Durham, NC, United States

Rett syndrome is a neurodevelopmental disorder caused by mutations in methyl CpG binding protein 2 (MECP2), a gene located on the X chromosome. Affected females carry one mutant copy and one normal copy in each of their

cells; however, due to X-chromosome inactivation (XCi), in any given cell, either the mutant copy or the normal copy is active, leading to mosaicism. The stochastic nature of X-inactivation results in a range of clinical severity, depending on the fraction of cells in the brain that have inactivated the mutant versus the wild-type copy of MECP2. This mosaicism, and the resulting heterogeneity in cellular phenotype, makes studies of MECP2 dysfunction in Rett Syndrome patients challenging.
In order to develop an in vitro model of Rett syndrome to study the involvement of MECP2 mutations in neural differentiation, function, and survival, we have generated iPSCs from dermal fibroblasts from 3 Rett syndrome patients. Several iPSC clones for each mutation were fully characterized for pluripotency and we determined the allelic expression of the MECP2 gene. These iPSC lines were also differentiated along a neuronal lineage and shown that all of them can become functional neurons.
X-chromosome inactivation often degrades over time during culture of iPSCs, resulting in cells that express both copies of X-linked genes, which complicates their use as models for X-linked diseases. To overcome this problem, we are using TALEN technology to target MECP2 in heterozygous female Rett iPSC lines to make paired isogenic iPSC lines that are either homozygous wild-type or mutated cell lines, in which MECP2 expression will not depend on XCi. These lines will be valuable tools to study the effects of the MECP2 mutations by comparing cells that are genetically identical except for the mutation. The ability to correct the mutation may also allow us to investigate the feasibility of gene therapy for treatment of Rett syndrome.

W-2131

GLOBAL GENE REGULATION BY METHYL CPG BINDING PROTEIN 2 (MECP2) IN PLURIPOTENT STEM CELLS

Yoshiaki Tanaka, Kun-Yong Kim, Mei Zhong, Xinghua Pan, Sherman M. Weissman, In-Hyun Park
Genetics, Yale School of Medicine, New Haven, CT, United States

Introduction
Rett syndrome (RTT) is one of the most prevalent female mental disorders. De novo mutations in methyl CpG binding protein 2 (MeCP2) are a major cause of RTT. MeCP2 regulates gene expression as a transcription regulator as well as through long-range chromatin interactions. Because MeCP2 is present on the X chromosome, RTT is manifested in a X-linked dominant manner. Murine MeCP2 null models and post-mortem human brain tissues have contributed to understanding the molecular and physiological function of MeCP2. In addition, RTT models using human induced pluripotent stem cells derived from RTT patients (RTT-iPSCs) provide novel resources to elucidate the regulatory mechanism of MeCP2. Previously, we obtained clones of female RTT-iPSCs that express either wild type or mutant MeCP2 due to the inactivation of one X chromosome. Reactivation of the X chromosome also allowed us to have RTT-iPSCs that express both wild type and mutant MeCP2. Recent studies showed that MeCP2 plays a critical role in astrocytes, microglia and mesenchymal stem cell, suggesting the critical role of MeCP2 in non-neuronal cells is important. Using our unique pluripotent stem cells, we investigated the regulation of gene expression by MeCP2 in pluripotent stem cells.
Results
We performed massively-parallel RNA sequencing (RNA-seq) in RTT-iPSCs from five different patients as well as normal ESCs/iPSCs. Although the expression of MeCP2 is relatively lower in pluripotent stem cell compared with neuron, our transcriptome analysis shows dysregulation of developmental genes in RTT-iPSCs. First, RTT-iPSCs expressing mutant MeCP2 lack several iPSC-specific genes, such as TMEM132D and FAM19A5. We also found that MeCP2 mutation induces overexpression of mitochondrial respiratory chain-related genes, resulting in reduction of apoptosis. In addition, even in pluripotent stem cell, MeCP2 mutation is involved in the regulation of genes related with neuronal development, but each mutation affects a partly different set of genes. Furthermore, loss of function in MeCP2 results in de-repression of genes on the inactive X chromosome.
Conclusion
These studies suggest that fundamental cellular physiology is affected by mutations in MeCP2 from very early fetal development and that a therapeutic approach targeting to unique forms of mutant MeCP2 is needed.

W-2132

PERSONALIZING THE STUDY OF INFECTIOUS DISEASE: MODELING HEPATITIS C VIRUS USING INDUCED PLURIPOTENT STEM CELLS

Robert E. Schwartz[1], Kartik Trehan[1], Linda Andrus[2], Timothy Sheahan[2], Alexander Ploss[2], Stephen Duncan[3], Charles Rice[2], Sangeeta N. Bhatia[1]

[1]Harvard–MIT Division of HST, Massachusetts Institute of Technology, Cambridge, MA, United States, [2]The Center for the Study of Hepatitis C, The Rockefeller University, New York, NY, United States, [3]Medical College of Wisconsin, Department of Cell Biology, Milwaukee, WI, United States

Human pathogens are responsible for 90% of human disease and 32% of deaths worldwide. Improved understanding of host-pathogen interactions will accelerate progress towards clinical therapy. The role that host genetics play in the natural history of infection and response to clinical therapy is poorly understood. Person-to-person variations in infection outcomes have long been appreciated, and genome-wide association studies are identifying predictive genetic signatures for these outcomes. Despite this progress, it is not generally possible to elucidate the interplay between genetics and infection due to the lack of model systems that capture host genetics. Achieving "personalized medicine" for infectious disease, whereby it would be possible to stratify patients genetically to make tailored clinical predictions and therapeutic recommendations, necessitates in vitro models that can systematically be sourced from genetic backgrounds of interest. We leverage induced pluripotent stem cells (iPSCs), which open the door to producing person-specific adult cell-types and tissues. We tested the feasibility of this experimental paradigm for a clinically relevant pathogen; hepatitis C virus (HCV) in stem cell derived hepatocytes. iPSC were differentiated towards hepatocyte-like cells (iHLCs) in a step-wise manner as exemplified by co-expression of multiple markers including albumin and alpha-1-antitrypsin, and secretion of liver-specific proteins. iHLCs were shown to express host factors important for HCV infection including CD81, SRB1, claudin1, and occludin. iHLCs infected with HCV demonstrate drug-sensitive viral replication. Transfer of supernatants from infected iHLCs to uninfected cultures showed the transmission of infection, verifying that infected iHLCs produce infectious virions and thus recapitulate the entire viral life cycle. Quantitative real-time PCR revealed that expression of inflammatory markers was up-regulated by HCV infection, demonstrating that HCV elicits a robust antiviral inflammatory response in iHLCs, a critical aspect of HCV pathogenesis in vivo. Finally, iHLCs derived from LDLR knockout patients had diminished infection compared to iHLCs derived from normal patients. This demonstrates for the first time that iPS and iPS derived hepatocyte-like cells can be used to explore the role that person-to-person variations play in HCV infection and more broadly viral pathogenesis.

W-2133

TCL1 OVEREXPRESSION HIGHLIGHT THE RELATIONSHIP BETWEEN PLURIPOTENCY AND TUMORIGENICITY

Tathiane M. Malta[1], Virginia Picanço-Castro[2], Daniel G. Pinheiro[1], Elisa M. Russo[1], Evandra S. Rodrigues[1], Rodrigo A. Panepucci[1], Dimas T. Covas[1], Simone Kashima[1]

[1]Univ of Sao Paulo, Ribeirao Preto, Brazil, [B]Regional Blood Center of Ribeirao Preto, [1]Univ of Sao Paulo, Ribeirao Preto, Brazil

Induced pluripotent stem cells (iPS) provide an unprecedented platform to model human disease, to screen for pharmacological molecules, and offer novel therapeutic strategy to generate patient-specific stem cell lines. However, iPS cells, and ESCs as well, have a tumorigenic potential that represents an obstacle to the safe use of pluripotent stem cell-based regenerative medicine therapies. Indeed, the relationship between tumorigenicity and pluripotency is largely unknown, but it seems that they are very related process.
With this in mind, the aim of this study was to analyze the contribution of TCL1A for reprogramming and if it plays a role in tumorigenicity. For this, we forced expression of TCL1A in fibroblasts and analyze their global expression profile.
We cultured the TCL1 transduced cells in embryonic stem cell condition. Interestingly, we observed a change in their morphology. The cells acquired a colony-like morphology with no defined edges. These cells were positive for

Nanog expression and negative for Oct4 and SSEA-4, by flow cytometry. The cells did not show alkaline phosphatase activity. Then, we used a microarray platform and found 3,491 genes (fold change greater than 2) that had been activated and repressed in the transduced cells compared to the donor fibroblasts. When we looked for the most represented molecular and cellular functions among the upregulated genes in TCL1-transduced cells, we found the following functions: cell cycle; cellular assembly and organization; and DNA replication, recombination, and repair. The upregulated genes included E2F1, E2F2, and CDK1. These finding suggest that TCL1 overexpression contributed for cell proliferation, which is important for pluripotency induction and for tumorigenicity as well. Although we observed SOX2 and SALL4 upregulation in TCL1-transduced cells, TCL1 alone was not able to activate pluripotency pathway.

When we take into account the downregulated genes in TCL1-transduced cells we found cellular movement; cellular development; and cell-to-cell signaling and interaction as the most represented functions. Cellular development encompassed genes related to epithelial-mesenchymal transition (EMT), including TWIST1, TWIST2, ZEB1, ZEB2, and SNAI2. We also observed downregulation of COL1A1, COL1A2, ACTA2, and MMP9. Together with CDH1 upregulation, these findings indicate that TCL1 overexpression contribute to EMT/MET process, which is related both to cancer and to pluripotency induction.

TCL1 was one of the 24 molecules tested by the first authors who derived iPS cells. It had been chosen because it is overexpressed during embryonic development. TCL1 is also overexpressed in human malignancies, which highlight the relationship of pluripotency and tumorigenicity. In fact, to induce pluripotency we need to induce self-renewal, which is also a property of tumors. The safety of stem cell regenerative therapies depends on how we will be able to control self-renewal and pluripotency and at the same time avoid tumorigenicity.

Although TCL1 seems not to contribute for pluripotency induction, it was able to induce molecular and cellular functions that are important for the reprogramming process, reinforcing the relationship between tumor and pluripotency.

W-2134

INCOMPLETE TELOMERE REPROGRAMMING UNDERLIES DEFECTIVE SILENCING OF EXOGENES IN PORCINE IPS CELLS

Guangzhen Ji[1], Kai Liu[1], Fang Wang[1], Sarantis Gagos[2], Lai Liangxue[3], Lei Xiao[4], Hongkui Deng[5], Ning Li[6], Lin Liu[1]

[1]Department of Cell Biology and Genetics, Nankai University, Tianjin, China, [2]Biomedical Research Foundation of the Academy of Athens Greece (BRFAA), Athens, Greece, [3]Guangzhou Institutes of Biomedicine and Health, Guangzhou, China, [4]Stem Cell and Developmental Biology Research Center, Zhejiang University, Hangzhou, China, [5]College of Life Sciences, Peking University, Beijing, China, [6]State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing, China

Sufficient telomere reprogramming and telomerase activation are critical for induction and self-renewal of pluripotent stem cells. Telomeres can be effectively reprogrammed and exogenous genes are fully silenced in mouse and human induced pluripotent stem (iPS) cells. However, exogenous genes remained active in most of porcine iPS cells generated in many laboratories thus far. We performed a systematic analysis of telomere length and maintenance of various porcine iPS cells generated from different cell types using various methods. We show that insufficient telomerase activation and incomplete telomere reprogramming were associated with many porcine iPS cell lines.

Porcine iPS cells at early passages (p5-10) with continuously activated exogenous genes exhibited telomere elongation and activation of telomerase to various degrees, compared to their progenitor cells, and their telomeres elongated or maintained during passages. Thus, sustained activation of exogenes appeared to improve telomerase activity and facilitate telomere elongation. In contrast, porcine iPS cells with silenced exogenous genes exhibited telomere shortening with increasing passages. Silencing or partial silencing of the exogenes was found in rare porcine iPS cells at early passages, yet the exogenes became reactivated during passages and this often was associated with insufficient activation of endogenous pluripotent genes and telomerase genes, leading to increased telomere damages and chromosomal instability. Moreover, porcine iPS cells had higher frequency

of telomere sister chromatid exchanges and t-circles that are involved in telomere lengthening by recombination. Nonetheless, they also showed increased frequency of telomere doublets, coincided with elevated telomere dysfunction induced foci, indicative of telomere damages. Notably, porcine iPS cells with reduced expression of pluripotent genes and telomerase genes exhibited increased frequency of DNA and telomere damages, which also increased with the passage numbers. Severe damages to DNA and telomeres led to elevated genomic instability as shown by abnormal karyotypes. The unique characteristics of porcine telomeres may partially explain failed telomere maintenance and reactivation of exogenes in porcine iPS cells during prolonged culture. Since authentic porcine ES cells still are not readily available as standard references for comparing the expression levels of pluripotent genes in porcine iPS cells, we speculated that exogenous genes remained to be activated to compensate for insufficiently activated endogenous pluripotent genes and to maintain telomeres in porcine iPS cells. Regardless of exogene inactivation, both telomerase-dependent and telomerase-independent mechanisms are involved in telomere reprogramming during induction and passages of porcine iPS cells. Further dissection of these pathways may help deeper understanding of telomere reprogramming and maintenance in porcine iPS cells. Also, the improvement in the induction method and culture conditions is required to fully activate endogenous genes and maintenance of telomeres, and thus will likely generate authentic pluripotent porcine iPS cells.

W-2135

REGULATION OF WNT SIGNALING DURING ANDROGEN RECEPTOR-MEDIATED APOPTOSIS IN BOVINE TESTICULAR INDUCED PLURIPOTENT STEM CELLS IN RESPONSE TO PHTHALATE ESTERS

Chia-Chen Ku[1], Ying-Chu Lin[1], Yukio Nakamura[2], Shigeo Saito[1], Kazushige K. Yokoyama[1]
[1]Koahsiung Medical University, Kaohsiung, Taiwan, [2]RIKEN BRC, Tsukuba, Japan

The induced pluripotent stem cells (iPSCs) were generated from bovine testicular cells by electroporation of Pou-domain transcription factor Oct4. Supplementation with leukemia inhibitory factor and bone morphogenetic protein 4 maintained and stabilized the expression of stemness genes and pluripotency. Bovine iPSCs displayed the normal karyotype and expressed alkaline phosphatase, SSEA-1, and SSEA-4. It expressed the stemness genes including OCT4, NANOG, MYC, KLF4, SOX2, STAT3, DNMT1, SUZ12, and MEF2A, and differentiated into cell types of all three germ layers in teratomas. We have also examined the effects of phthalate derivatives such as di-n-butyl phthalate (DBP), butylbenzyl phthalate (BBP), and di (2-ethylhexyl) phthalate (DEHP). The addition of DEHP repressed the expression of androgen receptor (*AR*) gene and the ability of iPSCs to commit to apoptosis. This was mediated by increased expression of $p21^{Cip1}$. DBP and BBP had no such effect. Loss of Wnt/β-catenin signaling, especially activities of the Frizzed receptor and Disheveled, was responsible for the effect of DEHP on AR-mediated apoptosis. Thus, the iPSCs from testicular cells are useful for screening the toxicity of environmental disruptors in terms of maintenance of stemness and pluripotency.

W-2136

ESSENTIAL ROLES OF TET1 IN MOUSE AND HUMAN PLURIPOTENCIES

Hidemasa Kato, Keiko Hiraki
Saitama Medical Univ, Hidaka, Japan

Mouse ESCs (mESCs) are largely accepted to have genuine self-renewing capacity and pluripotency. In contrast, human ESCs (hESCs) which are derived from the blastocyst but are more akin to the later epiblast-stage embryos show some resistance and biases toward differentiation. Moreover, human iPS cells (hiPSCs) tend to have lower self-renewal capacity when compared to mESCs and even to hESCs. Why all these cell populations differ this much? We here postulated that this could be because hiPSCs have not passed through a blastocyst-stage like other pluripotent stem cells (PSCs). We focused on proteins which expressions are present in blastocysts but not in the ensuing epiblasts. Tet1 was found to be such a protein. Our investigation indicated that Tet1 is involved in harnessing the full differentiation potential of mESCs. Without Tet1, the differentiation of mESCs decelerates which leads to a dif-

ferentiation resistance. Also in the mouse, Tet1 is only expressed up to the blastocyst stage. As the current method for deriving hiPSCs stands, we believe that hiPSCs would have little chance to ever express TET1, which may explain the differentiation defects of these cells. Therefore, we over-expressed TET1 in hiPSCs and its effects were studied. By comparing wild-type hiPSCs and TET1-overexpressing hiPSCs (TET1-hiPSCs), we found that the self-renewal ability has been improved upon TET1 expression. Next, we tested the pluripotency of the TET1-hiPSCs. When assigned to neuronal differentiation, these cells exhibited markedly higher efficiencies. Judging from the levels of SOX1, we found a 60-times increase in its efficiency when compared to wild-type hiPSC differentiation. We therefore concluded that the presence of TET1 reduces an inherent differentiation resistance of hiPSC, at least for the neural lineage, educing the bona fide pluripotency of these cells. An important implication is whereas “full” pluripotency may not strictly be necessary for life as judged by viable Tet1-KO mice, it is definitely needed for practical usages of PS cells. Our results are suggestive that Tet1 seems to be in charge of this “full” pluripotencies in both mouse and human PS cells and its absence in human pluripotency strongly damages its practical usages.

W-2137

IMAGE-BASED IRREGULAR IPS COLONY DETECTION FOR INTELLIGENT AUTOMATED CELL CULTURE

Megumi Matsumoto[1], Hiroto Sasaki[1], Risako Joto[2], Mika Suga[3], Masaki Kinehara[3], Kana Yanagihara[3], Yasujiro Kiyota[4], Kei Kanie[2], Hiroyuki Honda[1], Miho K. Furue[3], **Ryuji Kato**[2]
[A]Graduate School of Engineering, [B]Graduate School of Pharmaceutical Sciences, [1]Nagoya University, Nagoya, Japan, [2]Laboratory of Stem Cell Cultures, Department of Disease Bioresources, Research, National Institute of Biomedical Innovation, Ibaraki, Japan, [3]Nikon corporation, Tokyo, Japan

Pluripotent stem (PS) cell technology is the promising approach that widely expands the new approaches of pharmaceutical discoveries and regenerative medicine. However, including the recent progress of iPS cell researches, it still requires experienced technique to properly culture and maintains PS cells in stable undifferentiated status to provide reliable outputs in further usages. In spite of the accumulating knowledge of stem cell markers for evaluating the cellular status, it is widely known that the “colony morphology” is one of the most reliable markers to monitor or select the desirable PS cells. Commonly, PS cell colonies which clear and smooth edges with tight density is an aspect of desirable PS cell status, and colonies with “irregular morphology”, which consist of various types of irregular morphology, is known as the indication of undesirable PS cells. However, such morphological “definition by words” could not always fully reflect the information that used in experts’ recognition. Together with the biases of microscopic images, such as focus, brightness, view fields, timings, and etc., such image-based recognition was left in the black box of cell culture experts.
Here we propose a new methodology that combines automated cell culture observation system together with the colony morphology typing algorithm, which calculates the morphological feature data quantitatively, to automatically recognize the “irregular colony morphology” only from the images. Our data indicates that the morphological type that we recognize by our algorithm is detecting the irregular genetic profiles that arise in populations of iPS colonies.

W-2138

ENHANCED MAINTENANCE OF GENETIC INTEGRITY IN INDUCED PLURIPOTENT STEM CELLS

I-Chung Chen, John R. McCarrey
Biology, The University of Texas at San Antonio, San Antonio, TX, United States

During the past seven years, induced pluripotent stem cells (iPSCs) have revolutionized the stem cell field because these cells can be directly generated from various types of differentiated somatic cells using viral transduction or non-viral induction with pluripotency factor genes, RNAs or proteins, without the involvement of embryos. Thus, iPSCs are viewed as a preferable source of patient-specific pluripotent stem cells for uses in future clinical applications. However, the fact that iPSCs are derived from differentiated somatic cells raises certain concerns about the

safety of their use for therapeutic applications. One concern is that differentiated somatic cells typically bear a significantly greater mutational load than germ cells or early embryonic cells. Indeed, in our published and unpublished studies we have used the Big Blue Transgenic Mouse (BBM) system carrying the lacI mutation-reporter transgene to demonstrate that frequencies of spontaneous point mutations in differentiated somatic cells are significantly higher than those in germ cells, early embryonic cells, and embryonic stem cells (ESCs). This raises a question about the frequencies of spontaneous mutations in iPSCs and whether these will be more similar to the higher frequencies found in the somatic cells from which the iPSCs are derived or the lower frequencies found in other types of pluripotent cells which these cells resemble.
To test this question, we have generated iPSCs from mouse embryonic fibroblasts (MEFs) and adult tail-tip fibroblasts (TTFs) heterozygous for two different transgenes - 1) a drug-inducible cassette encoding four reprogramming factors, and 2) the BBM lacI mutation-reporter transgene. We obtained twelve MEF-derived, twenty TTF-derived, and six subcloned TTF-derived iPSC lines. All lines showed standard mouse ESC/iPSC colony morphology. Four MEF-derived, four TTF-derived and one TTF-derived subcloned iPSC lines were characterized for expression of mouse pluripotency markers, alkaline phosphatase, OCT4, SOX2, NANOG and SSEA-1, and found to be positive. Preliminary measurements of the frequency of spontaneous mutations in a population of MEFs and in an iPSC line derived from these MEFs indicate the mutation frequency in the iPSCs is significantly lower than that in the MEFs. Analyses of additional iPSC lines are underway. If we observe consistently lower frequencies of mutations in iPSCs than in the somatic cells from which they are derived, it will suggest that enhanced maintenance of genetic integrity occurs as a consequence of epigenetic reprogramming associated with converting differentiated somatic cells into iPSCs, consistent with our primary hypothesis that pluripotency and enhanced maintenance of genetic integrity are mechanistically linked at the genomic level.

W-2141

TAQMAN TRANSCRIPTOME ASSAYS FOR RAPID ASSESSMENT OF GENOME, EPIGENOME AND QUALITY OF INTEGRATION-FREE INDUCED PLURIPOTENT STEM CELLS

Uma Lakshmipathy[1], Rene Quintanilla[1], Andrew Fontes[1], Jeffrey Fergus[1], Candida Vaz[2], Vivek Tanavde[2]
*[1]Primary and Stem Cell Systems, Life Technologies, Carlsbad, CA, United States, [2]Bioinformatics Institute, A*STAR, Singapore, Singapore*

Pluripotent stem cells such as embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) are commonly identified and characterized based on biomarker expression. Current methods rely on a combination of in vitro and in vivo cellular methods to confirm pluripotency and tri-lineage differentiation potential. As the bottleneck in efficiency of reprogramming is alleviated with faster and better reprogramming systems, there is a need for high throughput characterization methods that allow for rapid confirmation of the quality of the resulting iPSC.
Molecular analysis platforms offer a quantitative, accurate and fast alternative to current methods and have recently been utilized to qualify pluripotent stem cells. Several platforms are available for gene expression analysis varying in content and complexity. To determine the optimal method and minimal set of genes required for definitive characterization of pluripotency, we have utilized high density array, medium and low density TaqMan® qPCR arrays to compare expression pattern of partially reprogrammed clones and fully reprogrammed iPSC in comparison to parental fibroblast and control embryonic stem cells. Results indicate that a focused set of genes in low and medium density arrays can recapitulate the information obtained with large scale arrays with distinct clustering of samples based on their pluripotency that correlated with cellular data. Further, this method was used to identify unique genes that were expressed differentially between partially reprogrammed cells and true iPSC clones as well as pluripotent cells and cells randomly differentiated via embryoid body formation.
Additional assays were carried in parallel to assess epigenome signature using TaqMan® Array Human MicroRNA Card and TaqMan® assays for copy number variation. Comprehensive analysis of the resulting data indicates similarities between the pluripotent clones but also detects subtle differences that can be further evaluated for their impact on functionality and long-term stability.

W-2142

GENERATION OF DISEASE-SPECIFIC INDUCED PLURIPOTENT STEM CELLS FROM POLYCYSTIC KIDNEY DISEASE PATIENTS

Wang Yang[1], Huang Jiahui[1], Tian Shoufu[1], Niu Xin[1], Hu Bin[1], Guo Shangchun[1], Chen Qinkai[2]

[1]Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China, [2]Department of Nephrology, The First Affiliated Hospital of Nangchang University, nanchang, China

Abstract

Introduction: Autosomal dominant polycystic kidney disease (ADPKD) is a common, monogenic multi-systemic disorder characterized by the development of renal cysts and various extrarenal manifestation, affecting approximately 7 million people worldwide. Mutations in the PKD2 gene is responsible for ADPKD in 15% patients. Recent studies have reported that induced pluripotent stem (iPS) cells hold promise to develop cell therapies and study disease mechanisms by genetic modification in vitro. So we selected the patients' differentiated fibroblasts with ADPKD for reprogramming of iPS cells line.

Methods: We screened a family with ADPKD, then obtained fibroblasts from the family members and a healty donor. These fibroblasts were infected by retrovious vectors encoding the human transcription factors (oct4, sox2, klf4 and c-myc) to generate iPS. Alkaline phosphatase staining was used to analyses the undifferentition phase of the ADPKD-iPS. Immunocytochemistry and Flow cytometry analyses were performed to test cell surface markers and pluripotency genes. Chromosomal G-band were used to analyses karyotype. Embryoid Body-mediatedand differentiation and teratoma formation demonstrated that iPS cells could differentiate into three germ layers in vitro/vivo. Sequence analysis of the whole PKD gene in the iPS line revealed a heterozygosity missense mutation.

Results: The ADPKD-ips cells exhibited morphology similar to human ES cells, characterized by large nuclei and scant cytoplasm with distinct borders . All of the ADPKD-iPS cells were positive for alkaline phosphatase . ADPKD-iPS cells were verified for the presence of cell surface markers (SSEA-4, TRA-1-60, TRA-1-81, Sox2, Oct4) and expressed pluripotency genes OCT4 by Flow cytometry analyses. Chromosomal G-band analyses demonstrated that iPS cells line had the same karyotype (46, XY) as the parent fibroblasts cells. Sequence analysis of the PKD2 gene confirmed a heterozygosity missense mutation: a GGG to GAA mutation at exon 1,which has not been reported before.

Conclusions: Our results demonstrate successful iPS cells generation from patients with a history of PKD gene mutation. iPS cells derived from ADPKD patients would provid a promising resource to study disease mechanisms, screen novel drug compounds, and develop new therapies.

Acknowledgement

This work was
supported by the National Natural Science Foundation of China (No. 81170640)
and by the Natural Science Foundation of Jiangxi Province (No. 2010GZY0333).

W-2143

GENERATION OF PORCINE IPS CELLS FROM ADIPOSE-TISSUE MESENCHYMAL STEM CELLS (PASCS) AND IMPROVED MAINTENANCE AND PASSAGING BY THE USE OF INACTIVATED PASC AS FEEDER

Diogo G. Biagi[1], Rafael Dariolli[1], Gustavo Mostoslavsky[2], Alexandre C. Pereira[1], Jose E. Krieger[1]

[1]Laboratory of Genetics and Molecular Cardiology, Heart Institute - InCor - University of Sao Paulo Medical School, Sao Paulo, Brazil, [2]Department of Medicine, Boston University School of Medicine, Boston, MA, United States

Adipose derived stem cells have been broadly used in cell therapy; however, they exhibit great limitations in their potential of differentiation to specific cell types. In contrast, a more robust differentiation potential may be achieved by reprogramming of somatic cells to a pluripotent state, such as induced Pluripotent Stem-Cells (iPSCs) capable of differentiating a variety of cell types. AIM: The aim of the present work was to generate porcine iPSCs (piPSCs) from Adipose-tissue mesenchymal Stem Cells (pASCs) and the evaluation of pASCs viability as feeder for the generated iPSCs. METHODS: pASCs extracted from an adult pig were grown until passage 5 and then transduced with a polysistronic lentiviral vector (STEMCCA). Two days after transduction, 2x104 cells were transferred to a 35 mm dish coated with Matrigel (BD Biosciences, NJ, USA) and cultured with E8 (Invitrogen, CA, USA) com-

plemented with 0.5 uM sodium butirate (Sigma, MO, USA). Medium was changed every other day until day 10 and every day until day 15-17. After picking, cells were cultured on matrigel coated dishes and medium was changed daily. RESULTS: On day 17, after viral transduction, colonies with morphology resembling human embryonic stem cells (hESCs) were manually picked. Nine of the 12 colonies selected grew until passage 4 and exhibited Alkaline Phosphatase (PA) activity. These colonies exhibited growth characteristics similar to hESCs, except for a higher rate of spontaneous differentiation (30%). After passage 4, two clones (piPSC-610 and piPSC-617) were selected to further expansion. We tested the expansion with two types of feeder: inactivated mouse Embryonic Fibroblast (mEF) at passage 5 and inactivated pASCs at passage 6. Feeders were plated at 0.4x103/mm2. Both clones decreased the spontaneous differentiation rate to 1-3%; however, pASC colonies took two passages to reach this value while mEF colonies took 4 passages. Moreover, colonies in pASC grew quicker and displayed lower rates of cell death and differentiation in the center of the colony compared to colonies grown in mEF. Finally, all colonies expressed molecular markers (RT-PCR for endogenous OCT4 and SOX2; NANOG, DNMT3b, and DPPA5) for the pluripotent state. Immunofluorescence results demonstrated that piPSCs expressed stem cell markers OCT-4, NANOG, SSEA-4 and TRA-1-60. Both clones formed embryoid bodies showing the markers of the three germ layers (NESTIN for ectoderm, DES for mesoderm and NCSTN for endoderm). CONCLUSION: We believe we provided the first evidence for porcine iPSCs generation from pASC and that pASC can be successfully used as feeder for piPSCs.

W-2144

MOUSE INDUCED PLURIPOTENT STEM CELLS TRANSPLANTATION RESCUE INJURED MOTONEURONS

Krisztian Pajer[1], Csilla Nemes[2], Sara Berzsenyi[2], Antal Nógrádi[1], **Andras Dinnyes**[2]
[1]Department of Ophthalmology, University of Szeged, Szeged, Hungary, [2]BioTalentum Ltd, Godollo, Hungary

Plexus injuries often result in the avulsion of one or more ventral roots, leading severe loss of motoneurons. Novel therapeutic approaches aiming to treat and alleviate the consequences of motoneuron death involve transplantation of stem cells, including induced pluripotent stem cells (iPSCs).
Here we report that mouse induced pluripotent stem cells are able to integrate in the host cord, rescue injured rat host motoneurons and promote their regeneration following transplantation into the spinal cord.
Mouse iPSCs generated by purified reprogramming protein (Klf4, Oct4, Sox2 and c-Myc) transduction were transplanted into the caudal part of the lumbar 4 (L4) segment of Sprague Dawley adult rats following the avulsion of the left L4 ventral root. The transplanted cells were mapped 5, 10 and 90 days after surgery using mouse specific antibodies to detect neuronal marker M6, astroglia marker M2 and stage specific embryonic antigen-1 (SSEA1).
In the transplanted animals significantly more motoneurons survived and innervated the denervated hind limb muscles compared with control animals. In the first 7 days after transplantation, the iPSCs expressed the neutrophic factors (GDNF and NT-4) and pro- and anti-inflammatory cytokines (MIP-1 alpha and IL-10). From this time onwards, iPSCs-derived neurons and astrocytes migrated throughout the L4 spinal segment. From day 10 after transplantation, the microglial activity in the graft increased and after day 16, the iPSCs and they derivatives disappeared completely from the spinal cord.
These data show that the mouse iPSCs differentiate into neurons and astrocytes in the injured spinal cord; express a set of molecules that promote the survival and regeneration of adult motoneurons after avulsion.
This study was financed by EU FP7 (PartnErS, PIAP-GA-2008-218205; Resolve, FP7-Health-F4-2008-202047; STEMCAM; PIAP-GA-2009-251186).

W-2145

C-FLIP PROTECTS HUMAN PLURIPOTENT STEM CELLS FROM TRAIL INDUCED APOPTOSIS

Vladimir Vinarsky[1], Jan Krivanek[1], Ladislav Andera[2], Ales Hampl[1]
[1]Masaryk University, Brno, Czech Republic, [2]Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic

Human pluripotent cells are extremely sensitive to DNA damage and undergo swift apoptosis upon DNA damage. Whereas expression of intrinsic apoptotic pathway was investigated, virtually nothing is known about expression and functionality of extrinsic apoptotic pathway in human pluripotent stem cells. We for the first time aimed to characterize expression and functionality of extrinsic apoptotic pathway. We focused on TNF Related Apoptosis Inducing Ligand (TRAIL) binding receptors in human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC).
Two hESC lines and one line of hiPSC were assayed for presence of membrane bound TRAIL receptors (DR4, DR5, DcR1, DcR2). Common pattern of expression of TRAIL receptors was observed among all investigated cell lines. Apoptosis inducing receptors DR4 and DR5 were found to be significantly expressed. We also found that intracellular components of extrinsic apoptotic pathway components namely caspases 3, 8 and 10, Bcl2 family BH3-only proteins (Bax, Bid) were found to be expressed in both hESC and hiPSC in comparable amounts. However when we tested ability of TRAIL to induce apoptosis we observed little activation of caspases and negligible increase in programmed cell death in both hESC and hiPSC alike. In order to dissect molecular mechanisms underlying observed TRAIL resistance cells were sensitized to TRAIL using proteo-synthesis inhibition. We found that increased sensitivity to TRAIL was accompanied by decrease of c-FLIP, inhibitor of caspase 8 activation, hinting at possible mechanism. We functionally tested role of c-FLIP by construction of cell lines with c-FLIP knockdown and found pronounced increase of apoptosis susceptibility upon TRAIL treatment in cells with decreased levels of cFLIP.
In this work we have for the first time mapped expression and functionality of TRAIL receptors and extrinsic apoptotic pathway in two types of human pluripotent stem cells- human embryonic stem cells and induced pluripotent stem cells. We found that irrespective of their origin human pluripotent stem cells are TRAIL resistant. Moreover we show that reduction of c-FLIP removes this resistance to TRAIL what indicates important role of c-FLIP in regulation of apoptotic signaling in human pluripotent stem cells.

W-2146

DEVELOPING A HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED NEURAL STEM CELL MODEL OF ENVIRONMENTAL IMPACT ON HUNTINGTON'S DISEASE REVEALS GENOMIC INSTABILITY DURING REPROGRAMMING

Andrew M. Tidball[1], Asad A. Aboud[1], Reed Chamberlin[2], Kevin K. Kumar[1], Mihir V. Odak[1], Bryan E. Cawthon[1], Kevin C. Ess[1], M. Diana Neely[1], Aaron B. Bowman[1]

[1]Neurology, Vanderbilt University, Nashville, TN, United States, [2]Genetics Associates Inc., Nashville, TN, United States

Large kindred studies have shown that environmental factors account for a large amount of variability in Huntington's disease (HD) age at onset. Our lab has generated human induced pluripotent stem cell (hiPSC) lines from control subjects and patients with Huntington's disease using episomal-vector based reprogramming methods. We have validated independent lines from each of 4 controls and 4 HD patients to confirm loss of the reprogramming vectors (PCR), normal genomic structure (karyotyping), conformity of microarray data to known hiPSC profiles (pluritest), and expression of pluripotency genes (qRT-PCR and immunostaining). During our stringent process of validating the quality of our lines, we found a statistically significant increase in the propensity for karyotypic abnormalities in lines generated from patients with HD versus controls. A large majority of the abnormalities we have seen are common to human pluripotent cell lines including trisomy 12 and i(20)(q10), but we also see inversions, translocations, and deletions not typical to these cells. The abnormalities were not accounted for by the genomic integrity of the fibroblasts used to generate the iPSCs. We use the Yamanaka et al (Kyoto University) published reprogramming protocol that includes a p53 shRNA, known to decrease genomic integrity during reprogramming. We are currently reprogramming the same cell lines without the p53 shRNA to test for a disease-dependent effect on the rate of karyotypic abnormalities.
We have also differentiated our hiPSCs into early striatal neural progenitors adapting methods developed by the Studer (Sloan Kettering Institute) and Zhang (University of Wisconsin) labs. Using a recombinant protein-free small molecule based protocol, we can differentiate cultures >90 % PAX6+/FOXG1+/ISL1+ confirmed by immunostain-

ing and quantitative RT-PCR from both control and HD hiPSCs. We are currently exploring the toxicological profiles of pathologically-relevant toxicants (heavy metals and pesticides) on the striatial neural progenitors differentiated from our control and HD hiPSCs as well as lines from individuals with mutations to the PARK2 gene. Increased sensitivity to a particular neurotoxicant provides evidence for a possible disease x environment interaction and permits future mechanistic studies examining toxicant mode-of-action and how it relates to HD pathology. In addition, we will also perform a meta-analysis comparing this study to a parallel study in floor plate neural progenitors generated from the same hiPSC lines treated with the same toxicants (See poster Neely et al).
Support: NIH F31 NS077632 and RO1 ES016931

W-2147

INDUCED PLURIPOTENT STEM CELLS REDUCE AIRWAY HYPERRESPONSIVENESS AND ALLERGIC REACTION IN SENSITIZED MICE

Shih-Jie Chou[1], Shih-Hwa Chiou[2]
[1]Pharmacology NYMU, Taipei, Taiwan, [2]Department of Medical Research & Education, Taipei Veterans General Hospital, Taipei, Taipei, Taiwan

B**ackground:** Allergic disorders have increased substantially in recent years. Asthma is characterized by airway damage and remodeling. Induced pluripotent stem cells (iPSCs) are embryonic-like cells derived from adult somatic cells by transduction of defined transcription factors. These cells have potential for multi-lineage differentiation and provide a resource for stem cell-based therapies. However, the therapeutic potential of iPSCs in allergic diseases and airway hyperresponsivenesss has not been investigated.**Objective:** The aim of this study was to evaluate the effect of iPSC transplantation on allergic reaction and airway hyperresponsiveness in a murine asthma model.**Methods:** BALB/c mice were sensitized with alum-adsorbed ovalbumin (OVA) and then challenged with aerosolized OVA. PBS or iPSCs were then intravenously injected after inhalation. Serum allergen-specific antibody levels, airway hyperresponsiveness, and cytokine production in spleen cells and bronchoalveolar lavage fluid (BALF) were then examined.**Results:** Treatment with iPSCs effectively suppressed both Th1 and Th2 antibody responses, which was characterized by reduction in serum allergen-specific IgE, IgG, IgG1, and IgG2a levels as well as in interleukin (IL)-5 and interferon-γ levels, and increased secretion of IL-10 in BALF and in OVA-incubated splenocytes. In addition, transplantation of iPSCs also significantly attenuated allergic airway hyperresponsiveness.**Conclusion:** Administration of iPSCs not only inhibited Th1 inflammatory responses but also had therapeutic effects on systemic allergic responses and airway hyperresponsiveness. We conclude that iPSC transplantation is a potential modality for treating allergic reactions and bronchial asthma.

W-2148

USING INDUCED PLURIPOTENT STEM CELL TECHNOLOGY TO MODEL IDIOPATHIC PULMONARY FIBROSIS

Daniel Sivalingam[1], **Preethi Vijayaraj**[1], Katherine Chung[1], Saravanan Karumbayaram[2], Zoran Galic[2], Brigitte Gomperts[1]
[A]Department of Pediatrics, [1]University of California Los Angeles, Los Angeles, CA, United States

Idiopathic pulmonary fibrosis (IPF) is a debilitating disease that is characterized by chronic, progressive, irreversible fibrosis of the lung interstitium. There is no treatment for this devastating disease except lung transplantation. Our scientific understanding of the biology and pathogenesis of IPF is severely lacking. It is likely the result of a complex interplay of genes and environment. Previous work has identified that the loss of lung epithelial cells together with the proliferation of fibroblasts that grow in their place is a major hallmark of IPF. It is unknown however whether the fibroblasts are causing the “die off” of lung epithelium or if the “die off” is caused by another mechanism and triggers the hyperproliferation of fibroblasts. These fibroblasts accumulate as foci that lay down excessive extracelluar matrix, causing excessive tissue remodeling, increasing the stiffness of the lungs thereby making it difficult for gaseous exchange. We believe that in studying the complex interplay between these two cells type that we may gain a great deal of insight into the pathogenesis of IPF and its progression. The most widely used bleomycin rodent model to study IPF results in fibrosis that is self-resolving, and therefore is not a relevant model of IPF. Induced pluripotent stem cells (iPSC) have been used to model other complex diseases including Alzheimer’s disease and Duchenne Muscular Dystrophy, where genes and environment play a role in disease pathogenesis; however, iPSC have not yet been used to model IPF or any other fibrotic disorder.
Our approach to advance our understanding of IPF is to utilize cellular reprogramming to model IPF in combination with a thorough genetic analysis of the disease. Our approach utilizes healthy donor and IPF patient-specific lung and skin tissue to generate induced pluripotent stem cells (iPSC) using the traditional four transcription factors.. We confirmed pluripotency of the iPSC lines by teratoma formation and immunofluorescence for pluripotency markers. In addition, all iPSC lines were found to be karyotypically normal.
Cells re-derived from spontaneous differentiation of iPSC’s were enriched for fibroblasts and the identity of the cells were confirmed using fibroblast / mesenchymal markers like CD44 and vimentin. We have successfully re-differentiated the iPSC’s from two IPF and control lines into fibroblasts. Remarkably, the re-derived fibroblasts from the IPF patient samples spontaneously generated fibrotic foci that were positive for a-smooth muscle actin and laid out excessive extracellular matrix as seen from trichrome staining. We are currently experimenting with culture conditions to replicate α-smooth muscle actin-expressing fibrotic foci in other IPF samples.
We are also generating alveolar type I and type II cells from the iPSC’s in order to be able to co-culture the epithelial and fibroblast cells to identify interactions between the cell types that could accentuate the phenotype in a dish. To this end, we successfully differentiated iPSC into definitive endoderm with 70% of cells expressing CXCR4 and generated anterior foregut endoderm with expression of TBX1 and PAX9. We are now developing methods to expand the lung bud Nkx2.1+ pool of cells to create lung epithelial progenitors.
Following the generation of a reproducible disease model, our next approaches are to use the model as a drug-screening platform and to gain further insights into the molecular mechanisms of the disease.

W-2151

GENERATION OF INDUCED PLURIPOTENT STEM CELLS WITHOUT SUPPRESSION OF p53

Nelson F. Lizier, Irina Kerkis
Genetics, Butantan Institute, Sao Paulo, Brazil

Induced pluripotent stem (iPS) cells appeared as an alternative to embryonic stem (ES) cell, which allowed to overcome ethical problems of embryo destruction and to obtain patient matched pluripotent stem cells for the future therapies. Our group isolated a unique neural crest-derived population of immature dental pulp stem cells (IDPSC) widely multipotent, not tumorigenic, which maintain their ‘‘stemness’’ for several serial passages in vitro. Here, we used IDPSC as an alternative source for iPS cells. IDPSC is a very attractive cell type that can be easily isolated from an assessable, privileged and non-painful source. Using four Yamanaka’s factors we easily derived iPS cells from IDPSC, which were named for IDPSC-iPSC. Reprogramming of IDPSC occurred under feeder-free condition, making simple pluripotent colony harvest and avoiding future problem of zoonosis. Such reprogrammed cells present all characteristics of human ES cells especially in respect of differentiation capacity. IDPSC-iPSC generated teratomas into nude mice demonstrating wide range of differentiated tissues. It was reported that suppression of p53 gene increase efficiency of reprogramming suggesting its use as an improvement tool. IDPSC strongly express p53, however they showed high reprogramming efficiency (2.8%). The suppression of p53 is dangerous because iPS cells, which may carry genetic aberration, but showing normal iPS cells morphology can be obtained. Thus IDPSC derived iPS

cells present advantageous cell type for future therapies since they maintain the expression of p53 suggesting that IDPSC-iPSC could be safer for application in stem cells therapies.

W-2152

CHARACTERIZATION OF iPS CELLS AND HEMATOPOIETIC CELLS FROM AN ICF SYNDROME TYPE2 PATIENT

Yutaka Yasui[1], Makoto Otsu[2], **Shin Kaneko**[1]

[1]Center for iPS cell Research and Application, Kyoto University, Kyoto, Japan, [2]Stem Cell Bank, Institute of Medical Science, The University of Tokyo, Tokyo, Japan

ICF syndrome is an extremely rare autosomal recessive disorder characterized by symptoms of immunodeficiency, chromosomal instability, and face deformity caused by DNA methylation abnormality. Defect of humoral immunity such as B lymphopenia with hypo γ-globulinemia is commonest immunodeficiency of the syndrome, followed by cellular immunodeficiency due to T- and/or NK lymphocyte dysfunction. Such combined immunodeficiencies frequently cause fatal opportunistic infections. DNMT3B gene mutation is known as only cause of ICF syndrome type I (ICF1). Recently, ZBTB24 gene was identified as a responsible gene of ICF syndrome type 2 (ICF2). We have established induced pluripotent stem (iPS) cells from a 40-year-old ICF2 male patient to clarify ZBTB24 function in hemato/lymphopoietic differentiation from induced pluripotent stem (iPS) cells.
iPS cell clones of an ICF2 syndrome patient were established from peripheral blood mononuclear cells (PBMC) by a sendai viral vector containing yamanaka-4-factors. Differentiation potential of the ICF2 iPS cells into hemato/lymphopoietic cell lineage was determined by co-cultivation method with suitable stromal cell. DNA methylation status at several hematopoietic stages was also determined.
Induction efficiency of ICF2 iPS cells by sendai-viral vector was equal to that of healthy volunteer's. Truncate mutation of ZBTB24 was confirmed in PBMC and iPS cells of the patient, but the specific chromosome aberration was detected only in PBMC. Though genomic DNA of ICF2 iPS cells were confirmed to be hypomethylated, their proliferation and teratomagenesis potentials were not different to normal iPS cells, but showed decreased sensitivity to radiation induced cell death. When they were differentiated into hematopoietic lineage, smaller number of hematopoietic stem cells (HSCs) can be obtained from ICF2-iPSCs. When they were stimulated by hematopoietic cytokines, large number of ICF2 HSCs stayed in G0 phase. Those results may suggest cell cycle associated role of ZBTB24 in hematopoietic differentiation.
Further cellular and molecular profiles of ICF2 iPS cells and their hemato/lymphopoietic derivatives will be discussed in the presentation.

W-2153

DIFFERENTIAL IMMUNOGENICITY OF CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Tongbiao Zhao
State Key Laboratory of Reproductive Biology, Institute of Zoology Chinese Academy of Sciences, Beijing, China

Tongbiao Zhao1,2, Zhen-ning Zhang1, Peter Westenskow3, Nongluk Plongthongkum4, Dinh Diep4, Zheng Hu5, Zhili Rong1, Meiyan Wang1, Dennis O Clegg6, Yong-guang Yang5, Kun Zhang4 , Martin Friedlander3 and Yang Xu1,*
1Division of Biological Sciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 920932; 2The State Key Laboratory of Reproductive Biology, Institute of Zoology Chinese Academy of Sciences, Beijing, 100101; 3Department of Cell Biology, The Scripps Research Institute, La Jolla, CA 92037; 4Department of Bioengineering, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093; 5Columbia Center for Translational Immunology, Columbia University Medical Center, New York, USA. 6Department of Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, Santa Barbara, CA 93106.
The advent of induced pluripotent stem cell (iPSC) technology has raised the possibility that patient-specific human iPSCs (hiPSCs) could become a renewable source of autologous cells for cell therapy without the concern for immune rejection.. However, the immunogenicity of cells derived from hiPSCs has never been examined. To rigorously address this issue, we optimized a humanized mouse model (denoted Hu-mice) reconstituted with functional hu-

man immune systems, which can mount vigorous T-dependent immune responses to human cell transplants. We demonstrate that these Hu-mice can effectively reject tissues derived from allogenic human embryonic stem cells (hESCs). By deriving integration-free hiPSCs autologous to the human immune system reconstituted in Hu-mice, we evaluated the immunogenicity of cells derived from hiPSCs with both teratomas assay and in vitro differentiated cells. Our findings indicate that different tissues derived from hiPSCs exhibit differential immunogenicity to the autologous human immune system.

W-2154

THE ACQUISITION OF SOMATIC MUTATIONS IN HUMAN INDUCED PLURIPOTENT STEM CELLS

Athurva Gore[1], Rui Liu[1], Athanasia Panopoulos[2], Ho-Lim Fung[1], Juan Carlos Izpisua Belmonte[2], Kun Zhang[1]
[1]Department of Bioengineering, University of California - San Diego, La Jolla, CA, United States, [2]Gene Expression Laboratory, Salk Institute for Biological Studies, La Jolla, CA, United States

Defined transcription factors can induce epigenetic reprogramming of adult mammalian cells into induced pluripotent stem cells (iPSCs). Recent studies have revealed that iPSC lines contain fixed point mutations after the process of reprogramming. However, the source of and mechanism behind these reprogramming-associated mutations remains unclear. It is unknown if mutations occur during the reprogramming process or are inherited from culture mutations in the progenitor cell population. Here we characterize in detail the origin of these mutations using a combination of whole-genome sequencing and targeted exome sequencing approaches. Using whole-genome sequencing data generated from small fractions of newly reprogrammed iPSC colonies, their three resultant iPSC lines, and their original progenitor cell line, we show that two-thirds of reprogramming-associated mutations pre-exist at low frequency in progenitor cells. We additionally demonstrate that pre-existing mutations and novel line-specific mutations are enriched for separate sets of epigenetic markers. We also show through repeated subcloning and targeted exome sequencing of three low-passage iPSC lines and one embryonic stem cell (ESC) line that iPSC genomes are relatively stable and uniform at the point mutation level during short-term culture, and that clonal expansion of single iPSCs does not inherently introduce mutations. Taken together, these results indicate that most iPSC mutations arise due to the culture of progenitor cells, and cannot be avoided using alternate reprogramming techniques. These results demonstrate the importance of genomic characterization of iPSCs prior to either in vitro or in vivo usage. Our findings will lead to a greater understanding of the origin of reprogramming-associated point mutations and will aid in the development of safe iPSCs for clinical use.

W-2155

PURIFIED DISTAL LUNG ALVEOLAR EPITHELIUM DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS REPOPULATE DECELLULARIZED LUNG SCAFFOLD

Mahboobe Ghaedi, Laura E. Niklason
Department of Anesthesia and Biomedical Engineering, Yale University, New Haven, CT, United States

It is postulated that the use of induced pluripotent stem (iPS) cells may be the most effective strategy to develop patient-specific respiratory epithelial cells that may be valuable in lung-related cell therapy and lung tissue engineering. To explore whether lung tissue can be regenerated in vitro, a relatively homogeneous population of alveolar epithelial type II (AETII) and type I cells (AETI) was generated from human iPS cells which had phenotypic properties similar to mature human alveolar type II and type I cells. Up to 97 % of cells were positive for surfactant protein C, 95% for Mucin-1, 93% positive for surfactant protein B, and the vast majority (89%) of the cells expressed the epithelial marker CD54. Additionally, more than 90% of AETI were positive for the type I marker, T1α and Caveolin-1. Acellular lung matrices were prepared by treating whole rat or human adult lungs with decellularization agents, and alveolar cells derived from human iPS cells were cultured on the acellular rat or human lung matrices in a lung bioreactor culture system. Under appropriate culture conditions, these progenitor cells adhered to and proliferated within a 3D lung tissue scaffold, displayed hierarchical organization within the matrix, and displayed multiple markers of differentiated pulmonary epithelium

W-2156

RESIDUAL UNDIFFERENTIATED CELLS DURING DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELL IN VITRO AND IN VIVO

Wei Fu[1], Shujun Wang[2], Wenjie Zhang[2], Guangdong Zhou[2], Wei Liu[2], Yilin Cao[2]
[1]Department of Pediatric Thoracic and Cardiovascular Surgery, Shanghai Children's Medical Center, Shanghai Jiao Tong Univ Sch of Medicine, Shanghai, China, [2]Shanghai Jiao Tong Univ Sch of Medicine, Shanghai, China

Induced pluripotent stem (iPS) cells are a potential cell source for regenerative medicine. However, the tumorigenicity of iPS cells is a big concern for clinical application. In addition to the genetic manipulation of the reprogramming process and the greater risk of tumor formation, it is unclear if iPS cells with normal development potential are still tumorigenic. Here we investigated three mouse iPS cell lines, including one line that is able to generate full-term mice via tetraploid blastocyst complementation. We found that a small number of undifferentiated iPS cells could be steadily isolated and expanded following long-term differentiation of cells *in vitro* or *in vivo*. The residual undifferentiated iPS cells could be expanded and re-differentiated, and undifferentiated pluripotent stem cells could again be isolated after further rounds of differentiation, suggesting that residual undifferentiated iPS cells could not be eliminated by extended cell differentiation. The residual undifferentiated cells could form teratomas *in vivo*, indicating that they are a potential tumorigenic risk during transplantation. These findings prompt us to reconsider the strategies for solving the tumorigenic problem of iPS cells, not only focusing on improving the reprogramming process.

W-2157

A HIGHLY REGERATIVE ANIMAL, PTYCHODERA FLAVA, REVEALS A ROLE IN REGENERATION FOR EVOLUTIONARY RELATIVES OF OCT4 AND NANOG.

Keith Weiser, Tom Humphreys
Institute for Biogenesis Research, University of Hawaii, Honolulu, HI, United States

Oct4 and Nanog are well known for their roles in maintenance and induction of a pluripotent state. In vertebrates, this activity is restricted to cells of the inner cell mass and primordial germ cells. These genes are also able to initiate a state of induced pluripotency in mature cells. We have identified candidate orthologs of Oct4 and Nanog in a highly regenerative hemichordate marine animal, *Ptychodera flava*. *P. flava* is notable for its ability to completely regenerate missing anterior and posterior body axes on bisection. No bilateral animal with a head and tail capable of extensive regeneration is more closely related to humans. The Oct4 candidate ortholog, PfPou3f3/4 shares 75% amino acid identity with human Oct4 within the conserved homeodomain and Pou domains. Allowing for conservative substitutions, the similarity is 85% over 127 amino acids and PfPou3f3/4 is the *P. flava* gene most similar to Oct4 and homologs from other species. Likewise, PfMsxlx is the P. flava gene most similar to vertebrate Nanogs. *In situ* hybridization of regenerating *P. flava* reveals expression of PfPou3f3/4 and PfMsxlx proximal to the blastema during regeneration and no expression in uninjured animals. The regenerating blastema presumably contains the pluripotent cells that will regenerate the missing body half and expression of orthologs of vertebrate pluripotency-associated genes may indicate a role for these genes in inducing or maintaining a pluripotent state during regeneration. PfPou3f3/4 is able to maintain expression of several Oct4-driven stem cell genes such as Zfp42 (Rex1), Fgf4, and Klf4 in mouse ES cells in the absence of Oct4 expression. Like Oct4, over-expression of PfPou3f3/4 reduces Wnt signaling in p19 embryonic carcinoma cells. Due to the sequence similarity and functional similarities, we believe that PfPou3f3/4 is an evolutionary relative of Oct4. We further hypothesize that PfPou3f3/4 has a function in reprogrammg cells to a multipotent or pluripotent state in regeneration. This may indicate that the reprogramming activity of pluripotency genes represents vestiges of a regenerative program that has been lost in vertebrates.

W-2158

REPROGRAMMING TO PLURIPOTENCY CAN CONCEAL SOMATIC CELL CHROMOSOMAL INSTABILITY

Liviu Malureanu, Masakazu Hamada, Tobias Wijshake, Wei Zhou, Jan van Deursen
Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, United States

One of the concerns regarding the safe use of induced pluripotent stem cells (iPSCs) in therapeutic applications is loss of genomic integrity, a hallmark of various human conditions and diseases, including cancer. Structural chromosome defects such as short telomeres and double-strand breaks are known to limit reprogramming of somatic cells into iPSCs. Reprogrammed cells have successfully been generated from somatic cells that undergo stable inheritance of an abnormal number of chromosomes, such as Down syndrome. This implies that aneuploidy (an abnormal number of chromosomes) is not a barrier to reprogramming. However, the extent to which defects that promote the continuous reshuffling of whole chromosomes during mitosis, a condition referred to as whole chromosome instability (W-CIN), interfere with efficient reprogramming of somatic cells is unknown.
To begin to address the impact of numerical chromosome instability on stem cells we examined the impact of two distinct W-CIN gene defects on somatic cell reprogramming.
We selected MEFs derived from *BubR1* (*BubR1*$^{H/H}$) and *RanBP2* hypomorphic (*RanBP2*$^{-/H}$) mutant mice for our studies because their aneuploidy rates are similar (36% and 33% aneuploid cells at passage 5, respectively) although the aneuploidization is driven by entirely different mechanisms. To induce reprogramming to pluripotency, Oct-3/4, Sox2, and Klf4 expression constructs were introduced in P5 wild-type, *BubR1*$^{H/H}$, and *RanBP2*$^{-/H}$ MEFs by retroviral transduction.
Here we demonstrate that MEFs with W-CIN gene defects fully reprogram to iPSCs with similar efficiency as wild-type MEFs but with highly contrasting outcomes on chromosome number integrity and stability: *BubR1* hypomorphic iPSC clones preferentially originate from aneuploid MEFs, while *RanBP2* hypomorphic iPSC clones preferentially stem from MEFs with normal diploid chromosome numbers, indicating that aneuploid cells can be selected for or against during reprogramming depending on the genetic defect driving the chromosome number instability. The observation that *BubR1*$^{H/H}$ aneuploid MEFs preferentially dedifferentiate raises the possibility that BubR1 might be a key component of a surveillance pathway that prevents aneuploid cells from reprogramming. Knowing that BubR1 levels decrease with aging, we hypothesize that reprogramming of somatic cells from elderly individuals into karyotypically normal and stable iPSCs may be particularly challenging. Our data uncovered the fascinating concept that a W-CIN gene defect (*RanBP2* hypomorphism) responsible for severe aneuploidization in somatic cells can become dormant upon reprogramming, indicating that testing of both iPSCs and the iPSC-founding cells for chromosome number instability will be necessary for the safe application of iPSC technology in regenerative medicine.

W-2161

THE DEVELOPMENT OF SMALL SCALE SINGLE-USE BIOREACTOR SYSTEM FOR HUMAN INDUCED PRULIPOTENT STEM CELL

Masanori WADA[1], Katsuhisa MATSUURA[2], Yoichi ISHIKAWA[1], Tatsuya SHIMIZU[2], Teruo OKANO[2]
[1]R and D section, ABLE Corporation, Tokyo, Japan, [2]Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan

[Introduction]
Induced pluripotent stem (iPS) cells are promising cell sources for regenerative medicine. The stirred suspension culture using a bioreactor system is an efficient method for the large scale expansion of human iPS cells. On the basis of our hypothesis that a low share stress and the uniform medium flow in a vessel are important for the suspension culture of human iPS cells, we have developed the bioreactor system which are capable of the stirred suspension culture of the inoculated single iPS cells in the aggregates condition. This system is also capable of operating eight 100mL bioreactors simultaneously, and controlling culture condition individually.
[Material and method]

At first the stirrer designs including pitched 6 blade impeller and delta shape paddle impeller were compared in terms of the hiPSC growth in suspension culture. The optical DO sensor and the small pH electrode were used in order to suppress the turbulent flow inside a reactor. The single cell suspension were prepared from 2D on-feeder culture and inoculated into 100mL bioreactor (2x10^5cells/mL). The cell aggregates were collected after the stirred suspension culture for 4 days, and dissociated into the single cells by using Accumax. Then the obtained single cells were re-inoculated into the bioreactor, and stirred suspension culture was performed up to 5 passages. The number of cells, and the number and size of aggregates were measured in every passage. The undifferentiated property of the expanded iPS cells was evaluated with the flow cytometric analysis.
[Results and discussion]
Although the culture using 6 pitched blade impeller failed to promote the aggregate formation and expand human iPS cells, the culture using the delta shape paddle impeller enabled to create a lot of aggregates 200 to 300 micrometer diameter in the cultivation for 4 days. Furthermore the number of cells increased 5 times (1x10^8cells) to the number of inoculated cells. The similar cell growth rate was observed for 4 days in each culture after the passage (1-5 passages). When obtained cell suspension was inoculated on the culture dish, hiPS cell specific colony formation was shown. The flow cytometric analysis revealed that more than 95% of population holded undifferentiated ability in all the passages. Collectively, we established the methods for the expansion of human iPS cells with undifferentiated state.

W-2162

GENERATION OF NEURONS FOR THE STUDY OF BIPOLAR DISORDER OF OLD ORDER AMISH PEDIGREE

Kwi Hye Kim[1], Rosamund Smith[2], Rachelle J. Galvin[3], Laurence Daheron[4], Steven M. Paul[5], Kalpana M. Merchant[1]
[A]Tailored Therapeutics-Neuroscience, [B]Regenerative Biology, [C]Quantitative Biology, [1]Eli Lilly and Company, Indianapolis, IN, United States, [2]Havard Stem Cell Institute, Harvard University, Cambridge, MA, United States, [3]Weill Cornell Medical College, New York, NY, United States

Bipolar disorder (BPD), also known as manic-depression, is a brain disorder manifested as unusual shifts in energy, activity level, and mood. According to the World Health Organization, bipolar disorder is the 7th leading cause of non-fatal burden in the world. Patients often show their first symptoms during late teens or early adult ages. The disease severely hinders their ability to perform daily tasks. Available treatments produce only partial responses and none cure the disease.
It is known that BPD runs in families, suggesting a role of genetic factors. However, the molecular mechanism(s) leading to BPD is poorly understood. To develop a cellular model that enables studies of molecular and functional mechanisms associated with BPD, fibroblasts from Old Order Amish pedigrees with high incidence of BPD and the emerging technology of induced pluripotent stem cells (iPSCs) have been utilized.
Fibroblasts from twelve individuals, representing six patients and a corresponding unaffected sibling, have been reprogrammed to iPSCs. Reprogrammed iPSCs have been characterized for their pluripotency. Established iPSCs were differentiated into neuroprogenitors and to neurons. Their differentiation states have been characterized by immunocytochemical analysis and quantitative real time polymerase reaction. The iPSC derived neurons here will be the foundation to study molecular mechanism of BPD in an *in vitro* model system.

W-2163

USING HUMAN IPSC-DERIVED NEURAL CELLS FROM GENETICALLY RELATED INDIVIDUALS TO UNDERSTAND BIPOLAR DISORDER

Jon Madison[1], Fen Zhou[2], Aparna Nigam[2], Ali Hussain[3], Doug Barker[4], Ralda Nehme[1], Karlijn van der Ven[1], Jenny Hsu[4], Steven Sheridan[5], Laurence Daheron[6], Roy Perlis[5], Pamela Sklar[7], Stephen Haggarty[8]
[1]Broad Institute/Harvard University, Cambridge, MA, United States, [2]Harvard University / Broad Institute, Cambridge, MA, United States, [3]Harvard University/Broad Insitute, Cambridge, MA, United States, [4]Broad Institute,

Cambridge, MA, United States, [5]Massachusetts General Hospital, Boston, MA, United States, [6]Harvard University, Cambridge, MA, United States, [7]Mount Sinai School of Medicine, Cambridge, MA, United States, [8]Broad Institute/ Massachusetts General Hospital, Boston, MA, United States

Bipolar disorder represents a significant burden to patients, families and society. Despite evidence for high heritability, the underlying etiology and pathophysiology of this psychiatric disorder remains poorly understood. The genetic landscape of psychiatric disorders is complex and existing approaches that model changes in small numbers of genes across development are not well suited to capture the multiple genetic factors interacting in an individual to cause disease risk. Advances in stem cell biology allow one to capture this genetic complexity in induced pluripotent cells (iPSCs) derived from patient fibroblasts and then differentiate them into disease-relevant cell types. This approach will enable the investigation of functional variation that contributes to risk for psychiatric disorders and the development of phenotype-based assays for use in high throughput small molecule and RNAi screens. To begin to develop an iPSC based platform to investigate psychiatric disorders, we reprogrammed a family of fibroblasts from a larger kindred segregating several forms of psychiatric illness including Type I bipolar disorder. We anticipated reprogramming a family would help control for phenotypic variability between individuals due to background heterogeneity. This family consisted of two healthy parents and their two bipolar sons. Our genetic analysis of these fibroblasts showed there were no gross genetic lesions. Subsequently, we derived 3 independent iPSC lines from each individual. Based on several criteria, including gene expression analysis (Pluritest) and teratoma formation, all 12 iPSC lines were shown to be pluripotent. From each iPSC line we used adherent monolayer neural induction cultures and fluorescence activated cell sorting to establish comparable populations of proliferative neural progenitor cell lines (NPCs). While we were able to establish three independent NPC lines from each of the unaffected parents, we were only able to establish NPCs from one out of three lines for each of the sons with bipolar disorder. Furthermore, the NPC lines we were able to establish from the bipolar sons showed decreased proliferation as assayed by BrdU incorporation. While each of the established NPC lines was capable of differentiating into neurons as defined by immunocytochemistry using lineage-specific markers, NPC lines derived from both bipolar disorder sons exhibited decreased numbers of neurons. We used several quantitative gene expression approaches to determine if the differences in establishment of NPCs were due to differences in differentiation capacity. We were unable to find a correlation between a cell line's ability to generate NPCs and a decrease in general differentiation capacity or neural differentiation capacity. Finally, we were able to rescue the proliferation defect of the bipolar disorder NPCs using a GSK3 small molecule inhibitor capable of activating the WNT pathway _ a known target of the mood stabilizer, lithium, which is used clinically to treat bipolar disorder. Taken together, our results suggest that these iPSC derived NPCs from bipolar individuals have a neurodevelopmental defect in the establishment or maintenance of iPSC-derived NPCs. We anticipate these approaches will provide novel insights into the etiology of these complex psychiatric disorders and paths toward novel targeted therapeutics for their treatment.
Research support provided by: NIMH (R33MH087896, R01MH091115), Stanley Medical Research Institute

W-2164

HUMAN IPSC-DERIVED MOTOR NEURONS, AN ATTRACTIVE MODEL FOR BOTULINUM NEUROTOXIN DRUG DISCOVERY

Morgan A. Hrones, Dejan Caglic, Tobin J. Dickerson
Chemistry, The Scripps Research Institute, La Jolla, CA, United States

Botulinum neurotoxin A, (BoNT/A) the causative agents for botulism, is documented as the most toxic proteins known to man, with a lethal dose of 1 ng/kg of body weight. Produced by the bacterium *Clostridium botulinum*, BoNT/A is currently regarded as one of the top six agents for bioterrorist use due to its long lasting effects of flaccid paralysis, relative ease in production, and ability to be aerosolized for mass intoxication in locations containing dense populations. In order to further our overall understanding behind this intracellular mechanism and to increase our accuracy with leads for future intracellular BoNT/A inhibitors, stem cells are an ideal model for BoNT/A intoxication studies. Stem cells, unlike other cellular models, produce large quantities of motor neurons and use quantities of BoNT representative of *in vivo* conditions. Thus, a suitable reporter has been prepared for detection of

BoNT/A intracellular activity containing the SNAP25 protein, the native substrate for BoNT/A, and two fluorescent proteins. These two fluorescent proteins allow the detection of BoNT intracellular activity via a change in fluorescence resonance energy transfer (FRET). Preliminary data has shown that two more traditionally used fluorescent proteins, namely CFP and YFP, are somewhat functional in cell lines, however, the low efficiency of FRET poses an issue for accurate detection. Thus, CFP and YFP have been exchanged with Clover and mRuby2, since this novel fluorescent pair has been documented to have higher FRET efficiency and dynamic range. After confirming the functionality of our reporter in a less sensitive model, namely a Neuro2A cell line, transfection of human induced pluripotent stem cells (hiPSCs) with the newly made construct allows us to engineer stem cells that give rise to motor neurons stably expressing our reporter of interest. Observing the actual cleavage of SNAP25 ultimately enables us to conduct small molecule screening campaigns with more accurate leads for future drug development that possess the ability to block intracellular activity of BoNT/A.

W-2165

AP-1 TRANSCRIPTION FACTOR JDP2 POTENCIATES THE GENERATION OF MEDULLOBLASTOMA CANCER STEM CELLS FROM INDUCED PLURIPOTENT STEM CELLS

Kazushige K. Yokoyama[1], Chia-Chen Ku[1], Jian-Yi Li[1], Nein-Chi Wu[1], Yu-Ting Yen[1], Shyh-Shin Chiou[2], Yukio Nakamura[3], Shigeo Saito[4]

[1]Graduate Institute of Medicine, Koahsiung Medical University, Kaohsiung, Taiwan, [2]Pediatrics, Koahsiung Medical University Hospital, Kaohsiung, Taiwan, [3]Cell Engineering Division, RIKEN BRC, Tsukuba, Japan, [4]Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

Transcription factor Jun dimerization protein (JDP2) plays roles in cell cycle regulation, cellular senescence, nuclear reprogramming and oncogenesis through the epigenetic control involved in cascades of $p19^{Arf}$-Md-m2-p53-p21-cyclin/CDK or $p16^{Ink4a}$-cyclin/CDK-RB-E2F. Clinical studies of medulloblastomas indicate that JDP2 might be a tumor suppressor gene candidate because normal granule cells express significant levels of JDP2, whereas cancer cells do not. Thus, we generated three different induced pluripotent stem cells (iPSCs) from human medduloblastoma cancer cells (DAOY1) using Lenti-virus encoded standard 4 factors (4F; Oct4, Klf4, Sox2 and c-Myc) and 2 factors (2F; Oct4 and JDP2). The original DAOY1 expressed three standard stemness genes like Oct4, Sox2 and Nanog, but did not show the alkaline phosphatase activity. By contrast, iPSCs expressed stemness genes and demonstrated the alkaline phosphatase activity. Moreover, we found that iPSCs enhanced the tumor progression as compared with DAOY1 in SCID mice. These results indicate that JDP2 is highly possible to play a critical role of tumor potentiating function of iPSCs to generate the cancer stem cells. We also found the role of JDP2 is concerned the signaling of Wnt signals such as the genes encoding LEF1 (lymphoid enhancer binding protein), TCF3. The cross talk of Wnt signal and LIF/JAK-STAT3-Oct4 will be critical for generation of cancer stem cells from medduloblastoma iPS cells.

W-2166

SANGER HUMAN INDUCED PLURIPOTENT STEM CELLS REPRESENT A DISTINCT PLURIPOTENT STATE WITH BIOLOGICAL CHARACTERISTICS SIMILAR TO MOUSE EMBRYONIC STEM CELLS

David J. Ryan, Wei Wang, Jian Yang, Fengtang Yang, Beiyuan Fu, Pentao Liu
Wellcome Trust Sanger Institute, Cambridge, United Kingdom

Introduction: From rodent studies it is clear that pluripotency is a dynamic state. Preimplantation and postimplantation embryos yield two distinct pluripotent states, naïve and primed respectively. To date, embryonic stem cells with characteristics of ground state pluripotency have not been derived from human embryos. Human embryonic stem cells seem to represent a pluripotent state more akin to the mouse primed state. Does the naïve state exist in non-rodent species? If so, then is the naïve state a transient metastable entity? How can we identify and capture this entity in humans? We sought to use cellular reprogramming as a means to interrogate this ques-

tion.Methods: Our published 6 factor reprogramming platform was used to generate Sanger Human induced pluripotent stem cells (SH-iPSCs) from human dermal fibroblasts. Reprogramming factors (OCT4, c-MYC, SOX2, KLF4, LRH1, and RARG) were delivered using episomal vectors or piggyBac mediated transposition of doxycycline inducible constructs. Generated lines were characterized for pluripotency gene / protein expression, loss of reprogramming plasmids / silencing of transgenes and *in vitro / vivo* differentiation. Parameters supportive of naïve pluripotency in mouse including X chromosome reactivation, immune privilege and biallelic nanog were tested.Results: Karyotypically normal, LIF dependent SH-iPSCs were produced which expressed the key pluripotency genes, had no detectable expression of exogenous factors and were competent for three germ layer differentiation. RNA FISH demonstrated two HPRT signals and no XIST cloud. Biallelic Nanog was also demonstrated on RNA FISH. During differentiation, reversibility was seen with appearance of XIST and loss of one HPRT signal. Expression profiling of X linked genes demonstrated dosage compensation during differentiation. Consistent with the immune privilege of the early embryo, SH-iPSCs had little MHC Class 1 expression suggesting a more immature phenotype.Conclusion: Sanger human induced pluripotent stem cells represent a distinct pluripotent state with properties similar to those of mouse embryonic stem cells. This cell type may be useful for studying early human development, including, the regulation of X chromosome inactivation during gastrulation.

W-2167

MODELING CFC SYNDROME USING PATIENT-SPECIFIC IPS CELLS

Kyu-Min Han, Yong-Mahn Han
KAIST, Daejeon 305-701, Korea, Republic of

Cardio-Facio-Cuteneous (CFC) syndrome shows various kinds of early developmental defects, including craniofacial dysmorphology, cardiac problem, mental retardation, and short statue. Most of CFC syndromes have mutations in RAS-MAPK signaling, thereby resulting in elevated ERK activity. However, little is known about how elevated ERK signaling is related to the patient's phenotypes at the cellular level. Here, patient-specific iPSCs were generated from dermal fibroblasts of CFC patient carrying c.770A>G mutation on BRAF gene by ectopic expression of OCT4, SOX2, NANOG, and c-MYC. CFC-iPSCs expressed pluripotent markers at transcription and protein levels with normal karyotypes. As a result, we found that abnormal embryoid body maintenance in CFC-iPSCs might be caused by mislocalization of beta-catenin. CFC-iPSCs will be useful for studying the early developmental defects of CFC syndrome.

W-2168

EPIGENETIC MEMORY OF IPS CELLS HELPS IMPROVE DIFFERENTIATION EFFICIENCY INTO RETINAL PIGMENT EPITHELIUM

Vaisakh Rajan, Janine Davis, Ruchi Sharma, Rafael Villasmil, Fang Hua, Qin Wan, Kiyoharu Miyagishima, Sheldon Miller, Kapil Bharti
NEI, NIH, Bethesda, MD, United States

Epigenetic Memory of iPS Cells Helps Improve Differentiation Efficiency into Retinal Pigment Epithelium
Vaisakh Rajan, Janine Davis, Ruchi Sharma, Rafael Villasmil, Fang Hua, Qin Wan, Kiyoharu Miyagishima, Sheldon S. Miller, and Kapil Bharti
National Eye Institute, National Institutes of Health, Bethesda, MD
Retinal degenerations are one of the leading causes of blindness in the US. Several forms of retinal degenerative diseases are caused by developmental or functional defects in the retinal pigment epithelium (RPE), a polarized monolayer of cells located in the back of the human eye. RPE functions to maintain the health and integrity of retinal photoreceptors, thus maintaining vision. Stem cell technology provides the possibility of developing *in vitro* disease models, cell-based therapies, and platforms for drug screenings. RPE can be differentiated from pluripotent stem cells. However, the differentiation efficiency is low and varies between different stem cell lines. We have used combination of a developmentally guided protocol and the “epigenetic memory” of induced pluripotent stem (iPS) cells to significantly improve the efficiency of differentiation into RPE. To harness the full potential of “epi-

genetic memory", we generated iPS cells from primary human fetal RPE (hfRPE) using the four pluripotentency factors SOX2, OCT3/4, KLF4, and c-MYC. These iPS cells were differentiated into RPE-like cells using a developmentally guided protocol that modulates TGF, WNT, FGF, and Nodal pathways. Differentiation efficiency of iPS cells derived RPE was checked using immuno-labeling and FACS analysis of three RPE-specific markers PAX6, MITF and TYROSINASE. The three markers are expressed in all the cells suggesting a differentiation efficiency of up to 100%. Our preliminary analysis shows that RPE derived from hfRPE derived iPS cells closely resemble primary RPE at molecular and physiological levels. We are currently performing a thorough molecular and physiological authentication of these cells. In conclusion, we have developed a protocol that efficiently differentiates iPS cells into RPE. This protocol will provide the basis for developing improved disease models and effective therapies.

W-2171

EFFECTS OF SOMATIC CELL OF ORIGIN ON NEURAL INDUCTION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Gunnar Hargus[1], Marc Ehrlich[1], Marcos J. Arauzo-Bravo[2], Jeong Beom Kim[3], Tanja Kuhlmann[1], Hans R. Schöler[2], Holm Zaehres[2]

[1]Institute of Neuropathology, Univsersity of Münster, Münster, Germany, [2]Max Planck Institute for Molecular Biomedicine, Münster, Germany, [3]Ulsan National Institute of Science and Technology, Ulsan, Korea, Republic of

Epigenetic memory in induced pluripotent stem (iPS) cells in regard to their donor cell type of origin could lead to variations in their differentiation capacities. We have generated iPS cells from human neural stem cells (NSC iPSC) and human cord blood derived CD34+ hematopoietic stem cells (CD34+ iPSC) as well as fibroblasts (Fib iPSC) and evaluated their potential to differentiate into neural precursor cells and into more mature neural cell types (Hargus et al., 2010, PNAS).
We performed whole genome expression analysis on enriched populations of nestin-positive and sox1-positive neural precursor cells from NSC iPSC, CD34+ iPSC and Fib iPSC and carried out an analysis of neural marker expression in terminally differentiated cells. Here, we found that neuronal differentiation was comparable and not significantly different between iPSCs derived from different somatic cell sources (NSC iPSC: 31,9%; CD34+ iPSC: 30,2%; Fib iPSC: 28,4% βIII-tubulin-positive neurons over all cells). In addition, we determined methylation patterns of neural genes in iPSC and human ES cells to decipher epigenetic memories.
Our data indicate that epigenetic differences in human iPS cell lines generated of different donor cell type of origin (ectodermal neural versus mesodermal blood cells or fibroblasts) seem to have moderate impacts on neural precursor cell generation, but do not have a significant impact on their terminal neuronal differentiation potential.

W-2172

MODIFICATIONS IN THE EXPRESSION OF HDACS AND DNMTS IN HUMAN NEURAL STEM CELLS INDUCED BY REPROGRAMMING AND LOW OXYGEN TENSION

Ilona Szablowska-Gadomska, Martyna Podobinska, **Leonora Buzanska**
Mossakowski Med Res Ct, Warsaw, Poland

Modifications in the expression of HDACs and DNMTs in human neural stem cells induced by reprogramming and low oxygen tension

Ilona Szabłowska-Gadomska, Martyna Podobinska, Leonora Buzanska
NeuroRepair Department, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland;
To better understanding the reprogramming and differentiation process in human neural stem cells we aimed to look at the mutual relationship between pluripotency regulatory network and epigenetic process at different oxygen conditions. Thus the expression of pluripotency genes, hypoxia inducible factors, histon deacetylases and DNA methyltransferases have been tested in HUCB-NSC (Human Umbilicar Cord Blood – Neural Stem Cell) line at diffrent developmental stages. Our previous data have shown that the low oxygen tension promote maintenance of undif-

ferentiated state of HUCB-NSC and is beneficial for their proliferation and activate Oct4 and Nanog genes in time of cultivation and developmental stage dependent manner. Cells incubated in all tested oxygen conditions expressed HIF 1 alpha and HIF 2 alpha, however expression of HIF 3 alpha was not detected. Elevated expression of HIF2α, but not HIF1α is correlated with the induction of pluripotency, but only after prolonged incubation of HUCB-NSC in low oxygen. It also enhanced neural commitment of HUCB-NSC as shown by elevated MAP2 accompanied by HIF1 expression. In this report genes involved in epigenetic modulations, such as HDAC1, HDAC2, DNMT3a and DMNT 3b, have been tested for their expression at different developmental stages of HUCB-NSC: in reprogrammed to pluripotency or kept in culture as neurally committed population. The induced pluripotent cells and neurally committed population have been both incubated in low oxygen tension condition. Our results show, that in neurally committed HUCB-NSC expression of HDAC2, DNMT3a and DMNT 3b was significantly lower than in pluripotent stem cells and ESC reference material. Induced pluripotent stem cells revealed significantly higher expression of two genes HDAC1 and HDAC2 as compared to ESC and differentiated cells. Our results did not indicated linear correlation between the expression of HDACs and DNMTs and cell developnental stages, however pluripotency regulatory network and epigenetic process at different oxygen conditions are highly interdependent.
Sponsored by grant from Polish MSRHE Nr N N302 597838, MMRC statutory funds and EIT Technology Park

W-2173

GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM CHARCOT-MARIE-TOOTH TYPE 2F PATIENTS AND DIFFERENTIATION INTO NEURONAL CELLS

Ji-Yon Kim[1], Jin-Mo Park[2], Young-Bin Hong[2], Kyung-Ha Ryu[3], So-Youn Woo[1], Byung-Ok Choi[2]
[A]Microbiology, [B]Neurology, [C]Pediatrics, [1]Ewha womans university, school of medicine, Seoul, Korea, Republic of

Charcot-Marie-Tooth (CMT) disease is a genetically and clinically heterogenous disorder causing chronic distal motor and sensory neuropathy. CMT type 2F is caused by point mutations of heat shock protein beta-1 (HSPB1) gene and their disease mechanisms are not fully elucidated yet. To overcome limitations of handing human neuronal cells in vitro, we generated CMT2F-specific iPSCs and differentiated into neuronal cells. iPSCs from normal controls and patients showed characteristics of colony morphology and expressed stem cell markers such as Nanog and SSEA4, and alkaline phosphatase. To verify functional pluripotency of stem cells, iPSCs were randomly differentiated into three germ layer cells in vitro. Then, iPSCs were directly differentiated into neuronal cells with retinoic acid (RA), sonic hedgehog (Shh), brian-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF). Here, we generated neurons derived from CMT2F-specific iPSCs for the first time which can be a prominent material to study disease phenotypes and drug screening.

W-2174

MODELING HEREDITARY HEMOCHROMATOSIS USING INDUCED PLURIPOTENT STEM CELLS.

Andreia Gianotti Sommer[1], Cesar A. Sommer[1], David Nunes[2], Gustavo Mostoslavsky[1]
[A]Center for Regenerative Medicine (CReM), [B]Medicine, [1]Boston University School of Medicine, Boston, MA, United States

Mutations in the HFE gene underlie the pathogenesis of Hereditary Hemochromatosis (HH), the most common inherited liver disease. We employed our STEMCCA lentiviral reprogramming vector to derive iPSCs from several patients with HH carrying distinct HFE mutations. The iPSC lines expressed the pluripotency markers SSEA-4, Tra-1-60 and Tra-1-81, displayed a normal karyotype, and were capable of efficient differentiation into hepatocyte progenitors expressing albumin, AFP, HNF4a and the HH-related genes HFE and hepcidin. Notably, HH-iPSC derived hepatocyte progeny expressed lower levels of hepcidin compared to normal cells, providing a potential experimental platform to study the disease phenotype in vitro. Furthermore, we engineered and validated Transcription Activator-like Effector Nucleases (TALENs) targeting the HFE locus in order to generate gene-corrected HH-iPSC lines for both disease modeling and cell replacement therapies.

W-2175

EFFICIENT GENERATION OF EQUINE MOTOR NEURONS FROM INDUCED PLURIPOTENT STEM CELLS.

Ruchi Sharma[1], David Hay[2], Xavier Donadeu[1]

[1]Developmental Biology, The Roslin Institute and Royal (Dick) School of Veterinary Studies, Edinburgh, United Kingdom, [2]MRC Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, United Kingdom

High fidelity models of neurogenesis are necessary to better understand mammalian development and disease. Equine motor neuron disease (EMND), comparable to the human disease, is currently untreatable and often results in euthanasia on ethical grounds. In contrast to humans, *in vitro* models of equine neurogenesis do not exist. Pluripotent stem cell populations have enormous potential in disease modelling given their ability to form derivatives of the three germ layers found in the developing embryo. While a promising notion, the isolation of equine embryonic stem cells has thus far proved elusive and therefore it has been necessary to explore other alternatives to generate pluripotent stem cell populations. Induced pluripotent stem cells (iPSCs) are generated by reprogramming somatic cells to an embryonic state. Therefore iPSCs represent an extremely valuable tool for modelling disease and organ toxicity, with enormous potential in veterinary medicine. In this study, we describe the generation of iPSCs from equine keratinocytes and their efficient and robust differentiation into motor neurons.
Skin biopsies from a 5-month old foal were used to derive keratinocyte cultures. These cells were then transduced with retroviral constructs coding for murine Oct-4, Sox-2, c-Myc and Klf-4 sequences, following the original Yamanaka protocol. Following transduction, tight cell colonies were generated that had sharp boundaries, stained positive for alkaline phosphatase and resembled previously reported human iPSCs. The reprogrammed cells were successfully maintained in feeder free and serum free conditions with LIF supplementation for 40 passages. Immunochemistry and qPCR analyses revealed the iPSCs lines expressed pluripotency markers expressed in equine embryonic stages including, Oct4, Sox2, SSEA1, Lin28, Nanog, Rex1, Tert and Dnmt3b. Equine iPSC*s* were able to form embryoid bodies and to differentiate into derivatives of the three germ layers *in vitro* as indicated by expression of lineage-specific markers including alpha-Fetoprotein (AFP) and Gata-4 (endodermal), Nestin and Neuron specific class III Beta Tubulin (Tubb3) (ectodermal), and vimentin and smooth muscle actin (mesodermal). Equine iPSCs were pluripotent *in vivo* as demonstrated by the formation of teratomas consisting of tissue derivatives of all three lineages such as bone, cartilage, pulmonary epithelium and mature neurons in SCID mice. Importantly, in addition to its ability for spontaneous differentiation, equine iPSCs were amenable to efficient and robust directed differentiation into neuronal tissue. Equine iPSCs were successfully induced to differentiate into neurospheres forming extensive neuronal projections and synapses which stained for neuronal markers including Tubb3, Map2, Synapsin1 and Choline acetyl transferase (ChAT). In addition, gene expression analysis with qPCR revealed expression of Foxg1, Pax6 and Nestin during induced neurogenesis, followed by up regulation of ISL1, a potent and specific inducer of motor neurons, during terminal differentiation. In conclusion, we report for the first time the generation of equine neurons in vitro, providing, also for the first time, demonstration of the potential of iPSCs in equine biomedicine. The ability to derive motor neurons *in vitro* opens the way for new and exciting applications in equine regenerative medicine and reveals highly conserved pathways of differentiation across species.

W-2176

GENERATION OF INDUCED PLURIPOTENT STEM CELLS (IPSC) FROM PATIENTS WITH FAMILIAL OSTEOCHONDRITIS DISSECANS (FOCD) TO LINK DEFECTIVE MATRIX ASSEMBLY WITH CARTILAGE INJURY

Maojia Xu[1], Georgina Shaw[1], Eva-Lena Stattin[2], Dick Heinegard[3], Alan Colman[4], Mary Murphy[1], Frank Barry[1]

*[1]Regenerative Medicine Institute (REMEDI), National University Ireland, Galway, Galway, Ireland, [2]Department of Medical Biosciences, Umeå University, Umeå, Sweden, [3]Department of Clinical Sciences Lund, Lund University, Lund, Sweden, [4]A*STAR Institute of Medical Biology, Singapore 138648, Singapore*

Background

Familial osteochondritis dissecans (FOCD) is an autosomal dominant connective tissue disorder characterised by disturbed chondro-skeletal development, disproportionate growth and deformation of the skeleton in affected family members. A recent study indicated that a missense mutation in the aggrecan C-type lectin domain is responsible for impaired matrix assembly and function in FOCD cartilage.

Objective

Previously, attempts have been made to isolate bone marrow derived mesenchymal stem cells (MSCs) from FOCD patients in order to study the organisation of extracellular matrix during chondrogenesis. The isolation was difficult and yields of MSCs were low. Therefore the objective of this study was to generate FOCD specific iPSC-derived MSCs to use as a study model for FOCD.

Methods

FOCD-iPSC lines were generated from skin fibroblasts of two FOCD patients by ectopic expression of Yamanaka Factors (Oct4, Sox2, Klf4, c-myc) that were encoded in retroviral vectors. To assess the pluripotency of induced hESC-like colonies, semiquantitative RT-PCR, immunocytochemistry, flow cytometry, teratoma formation in vivo and karyotype analysis were performed. Two non-patient iPSC lines were used as positive controls in all of the above experiments.

The disease phenotype was characterised in FOCD-iPSC lines by DNA sequence analysis in order to validate the disease related mutation. To generate the iPSC-derived FOCD model, FOCD-iPSCs were differentiated into MSC-like cells by isolating and expanding the outgrowth cells from the embryoid bodies (EBs). Positive and negative makers for MSCs were analysed by flow cytometry. Multipotency was assessed by chondrogenesis, adipogenesis and osteogenesis assays. Alizarin Red staining and Oil red O staining were used to detect calcium and fat in the differentiated cultures. Toluidine blue staining and dimethyl methylene blue (DMMB) assay were performed to detect acidic proteoglycan and to quantify GAG production in chondrogenic pellets.

Results

Seven hESC-like colonies were obtained from donor 1 and three hESC-like colonies were obtained from donor 2. Two colonies were randomly selected to represent each patient, named as FOCD1-2, FOCD1-30, FOCD2-9 and FOCD2-13. These colonies were expanded in culture, characterized then differentiated. The results of characterization of FOCD-iPSC lines indicated that retroviral reprogramming factors were silenced in all the cells and each FOCD-iPSC displayed a normal karyotype. Secondly, endogenous pluripotency markers (OCT4, SOX2, NANOG, SSEA4 and TRA1-81) were positively expressed in iPSCs. iPSCs were injected into SICD mice, after 6 weeks all of cells were able to form teratomas containing tissues from the three germ layers. In addition, the disease relative mutation was found to remain in all four patients' iPSCs.

For the generation of iPSC-derived FOCD model, iPSCs were differentiated into MSC like cells. The analysis of flow cytometry showed that the majority of the FOCD-MSC preparations were CD73, CD90 and CD105 positive and CD19, CD34 and HLADR negative. In addition, differentiation assays indicated that all FOCD-iPSC-derived MSC like cells were capable of differentiating into chondrocytes, osteoblasts and adipocytes.

Conclusion

We have successfully generated iPSC-derived FOCD model. Further studies will focus on investigating the molecular role of the mutation of aggrecan C-type lectin domain in the etiology of FOCD.

W-2177

THE EFFECT OF DONOR AGE ON THE INDUCTION OF PLURIPOTENCY IN BONE MARROW DERIVED MESENCHYMAL STEM CELLS FROM YOUNG AND AGED INDIVIDUALS

Matthias Megges[1], Sven Geissler[2], Patrik Stumpf[3], Richard Oreffo[3], James Adjaye[4]

[1]Molecular Embryology and Aging Group, Max Planck Institute for Molecular Genetics, Berlin, Germany, [2]Julius Wolff Institut, Campus Virchow-Klinikum, Berlin, Germany, [3]Faculty of Medicine, University of Southampton, Southampton General Hospital, Southhampton, United Kingdom, [4]Institute for Stem Cell Research and Regenerative Medicine, Heinrich Heine University Duesseldorf, Duesseldorf, Germany

Human bone marrow derived mesenchymal stem cells (hBM-MSCs) are a multipotent cell type capable of differentiating into osteoblasts, chondrocytes and adipocytes. However, the in vitro expansion and application of primary human mesenchymal stem cells are limited by their short life span in culture and restricted differentiation potential. These two features have been shown to be altered in somatic cells by reprogramming them to induced pluripotent stem cells (iPSCs), thus making iPSC derivation a promising approach to broaden the potential use of hBM-MSCs. Because iPSCs have great potential in regenerative therapies for age-related diseases, it is crucial to investigate reprogramming mechanisms within the context of hBM-MSCs from aged individuals in order to identify age-associated obstacles. Differences in metabolism, elevated levels of reactive oxygen species (ROS), proliferation, genome instability and senescence have been shown to be associated with increased donor age in hBM-MSCs. Age-relate features and their role in reprogramming have been analysed in a limited number of studies in human fibroblasts. However, little is known about the role of age-associated molecular changes during reprogramming of hBM-MSCs and the effect on the derived iPSCs. To obtain new insights into the potential roles of age related features in reprogramming of hMSCs to iPSCs we induced pluripotency in hBM-MSCs from fetal femur (55 days post conception) and hBM-MSCs of aged donors (60-70 years of age) via the classical retroviral and the non-integrating episomal plasmid based reprogramming approach. Higher levels of ROS, phosphorylated γH2AX and slower proliferation could be detected in hBM-MSCs from aged individuals. Karyotype abnormalities were not detected in BM-MSCs of both groups. Microarray-based comparative transcriptome analyses identified genes involved in p53 and hypoxia pathways, cell cycle regulation, steroid biosynthesis and glutathione metabolism as down-regulated in hBM-MSCs from aged donors in comparison to fetal femur hBM-MSCs. However, genes involved in focal adhesion, the lysosome and glycolysis pathways were up-regulated in hBM-MSCs from aged donors. Whether the identified age related features in hBM-MSCs are altered during the reprogramming process and if they persist in the respective iPS cells derived from them is under investigation. Human BM-MSCs from fetal femur could be reprogrammed more efficiently and faster compared to BM-MSCs from aged donors using both the retroviral and episomal plasmid-based reprogramming methods. We have derived a fully reprogrammed iPSC line from BM-MSCs from an aged donor (60 years of age) using retroviruses. Three fully reprogrammed iPSC lines have also been derived from fetal femur BM-MSCs using the episomal plasmid-based approach. However, episomal-based reprogramming of BM-MSCs from aged donors did not give rise to fully reprogrammed iPSCs. One aged hBM-MSC line gave rise to partially reprogrammed iPSC clones. In conclusion we have demonstrated that the efficiency of inducing pluripotency in hBM-MSCs is potentially affected by the age of the donor and that age-associated features such as elevated ROS levels, altered transcriptomes and cell cycle regulation diminish the pace and efficiency of inducing pluripotency in BM-MSCs from aged donors. We anticipate that our current findings might help identify and overcome age-associated roadblocks inherent to the derivation of iPSCs from hBM-MSCs.

W-2178

IMPROVED REPROGRAMMING EFFICIENCY OF DISEASE SPECIFIC iPSC USING IMMORTALIZED HUMAN FORESKIN FIBROBLAST FEEDER CELLS

Pooja Teotia[1], Sujata Mohanty[1], Madhulika Kabra[2], Shefali Gulati[2], Balram Airan[1]
[A]Stem Cell Facility, [B]Pediatrics, [1]AIIMS, New Delhi, New Delhi, India

Generation of disease specific Induced Pluripotent Stem Cells (iPSC) represents a clear breakthrough in regenerative medicine. Hence, it is of great interest to establish optimal feeder and culture conditions that allow successful derivation & propagation of iPSC in clinically compatible conditions. Feki et. al. have established the Immortalized Human Foreskin Fibroblasts (I-HFF) feeder with an added advantages of endogenous bFGF secretion which at the same time supports the successful propagation of human Embryonic Stem Cells (ESC) and iPSC. In this study we aim to compare the derivation and propagation of iPSC using mouse feeder along with I-HFF feeder. Human skin fibroblast cells obtained from Duchenne Muscular Dystrophy patients, were used to derive iPSC cells using polycistronic lentiviral vector.
Study was initiated after the approval from Institute Ethics Committee (IEC) & Stem Cell Ethics Committee. In vitro-culture and expansion of fibroblasts cells were established using explant method. 1x 105 Fib cells were infected with hSTEMCCA (Human Stem Cell Cassette) lentiviral vector in the presence of 5ug/ml Polybrene. Next day, cells

were plated onto mouse and I-HFF feeder at a density of 1x104 cells onto the 6- well plate. After 24 hours, the medium was switched to reprogramming medium (ESC media and I-HFF conditioned media in 1:1 ratio).
Induced colonies were picked up based on human ES cell colony morphology and live staining for TRA-1-60 marker at days 16-24 post infection. The iPSC lines were assessed on the basis of morphology, expression of pluripotent makers by Immunoflorescence & RT-PCR. The in -vitro pluripotency and ability to differentiate into three germ layers was assessed by embryoid body formation. The experiments were performed using fibroblast cells from two different patients in triplicates.
We found that the average days of appearance of colonies was 16 on human feeder in comparison to 24 on mouse feeder. The total number of colonies obtained from two independent experiments at the end of reprogramming period was 41 and 23 on human and mouse feeders respectively. The average reprogramming efficiency of iPSC on human vs mouse feeders were 0.1 % and 0.05 % respectively as demonstrated by TRA 1-60 Live staining. Disease specific DMD-iPSC generated in this manner displayed ES cell like morphology, expressed stem cell markers TRA 1-60 and TRA 1-81. These iPSC ines exhibited endogenous expression of pluripotency markers like OCT-4, Sox2, Klf-4, cMYC and Nanog. The iPSC lines derived using both the feeder cells were able to spontaneously differentiate into cells of all three germ layers as characterized by Immnunofluorescence and RT-PCR assay.
Cumulatively, our study demonstrates that the (i) I-HFF human feeder was more efficient for the derivation of iPSC as evident by maximum number of fully reprogrammed clones as comparison to Mouse feeders, (ii) the emergence of iPSC clones took less reprogramming time on human feeders versus mouse feeders.The above observations can be attributed to the characteristic endogenous bFGF secretion by I-HFF feeders.
I-HFF cells at present are the best and economical candidate feeder cells for maintaining hESC/iPSC in culture as they minimize batch to batch variation and limiting the addition of exogenous bFGF.
Acknowledgment: We thank Prof. Anis Feki for providing us I-HFF cell lines.

W-2181

MESENCHYMAL STEM CELL-LIKE CELLS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS ENHANCE PERIODONTAL REGENERATION

Kim Hynes[1], Danijela Menicanin[1], Jennie Han[1], Victor Marino[1], Krysztof Morzik[1], Stan Gronthos[2], Mark Peter Bartold[1]

[1]Colgate Dental Research Centre, The University of Adelaide, Adelaide, Australia, [2]School of Medical Sciences, Mesenchymal Stem Cell Laboratory, University of Adelaide, Australia

Mesenchymal stem cells (MSC) have received significant attention in recent years due to their suitability for use in regenerative medicine. The therapeutic potential of MSC has however highlighted the need for identifying easily accessible and reliable sources of these cells. Current procedures for obtaining MSC are invasive, expensive and laborious. An attractive alternative source for obtaining large populations of MSC is through controlled differentiation of readily expandable induced pluripotent stem cells (iPSC).
We have established a protocol for inducing human iPSC to differentiate into MSC. MSC like cells were generated by differentiating iPSC in MSC media for two weeks followed by serial passaging to select for fast growing MSC like cells which have the capacity to attach and proliferate in mono-layer cultures, whilst eliminating slow-growing differentiating iPSC. Using this protocol MSC like cells were generated from iPSC lines derived from three different somatic tissues; gingiva, periodontal ligament, and foreskin. The resulting MSC like cells generated express key MSC-associated markers including (CD73, CD90, CD105, CD146 and CD166) and lacked expression of pluripotent markers (TRA160, TRA181 and alkaline phosphatise) and hematopoietic markers (CD14, CD34 and CD45). In vitro the iPSC-MSC like cells displayed a differential capacity to differentiate into osteoblasts, adipocytes and chondrocytes. In vivo subcutaneous implant of the iPSC-MSC like cells into NOD/SCID mice re-confirmed the differential differentiation capacity between the three iPSC-MSC like cells lines which was evident in the in vitro differentiation. When these iPSC-MSC like cells were implanted into periodontal defects in immunocompromised rats a significant increase in the amount of regeneration and newly formed mineralised tissue compared to control rats was noted.

Together these findings demonstrate that MSC like cells can be generated from iPS cells and could provide a novel and unlimited source of MSC cells which will be much safer for use in periodontal regenerative therapy.

W-2182

SPECIAL AT-RICH BINDING PROTEIN SATB1 CONTROLS RE-ORGANIZATION OF LINEAGE-SPECIFIC DIFFERENTIATION PROGRAMS DURING KERATINOCYTE REPROGRAMMING TOWARDS THE INDUCED PLURIPOTENT STATE

Tatyana Y. Sharova[1], Mohammed I. Ahmed[3], Hye-Jung Han[4], Krzysztof Poterlowicz[3], Gustavo Mostoslavsky[2], Terumi Khowi-Shigematsu[4], Vladimir A. Botchkarev[3], **Andrey A. Sharov**[1]

[A]Dermatology, [B]Department of Medicine, [1]Boston University School of Medicine, Boston, MA, United States, [2]Centre for Skin Sciences, University of Bradford, Bradford, United Kingdom, [3]Lawrence Berkeley National Laboratory, Berkeley, CA, United States

Reprogramming of adult somatic cells towards the induced pluripotent state (iPS) is associated with marked changes in the genome organization and gene expression programs. Genome organiser and special AT-rich binding protein Satb1 plays an important role in the control of higher-order chromatin remodelling in a number of lineage-specific gene loci including keratinocyte-specific genes, which is required for maintenance of their transcriptionally active status. Here, we show that during reprogramming of primary epidermal keratinocytes isolated from transgenic mice expressing the Dox-inducible cassette of four mouse pluripotency genes (*Oct4, Sox2, Klf4* and *c-myc*) under *Col1a1* promoter, the expressions of Satb1 and keratinocyte-specific genes that constitute keratin type I and type II loci, as well as Epidermal Differentiation Complex locus, are markedly downregulated, while expression of the pluripotency gene Nanog is upregulated. ChIP analyses revealed Oct4 binding to regulatory regions of the *Satb1* gene indicating that pluripotency factors might directly repress Satb1 expression during reprogramming. Furthermore, treatment of keratinocytes with lentiviruses expressing Satb1 shRNA significantly accelerated appearance of Nanog+ cells and iPS colonies compared to the controls. Acceleration of iPS colony formation under shRNA-mediated Satb1 knockdown was accompanied by more rapid decrease in the expression of keratinocyte-specific genes, compared to controls. Thus, these data demonstrate that Oct4-mediated downregulation of Satb1 serves as an essential step in switching-off keratinocyte-specific gene expression programs during their reprogramming towards the induced pluripotent state

W-2183

SOMATIC MOSAICISM IN HUMAN SKIN FIBROBLASTS REVEALED BY WHOLE GENOME SEQUENCING OF INDUCED PLURIPOTENT STEM CELLS

Alexej Abyzov[1], Alexander Eckehart Urban[2], Mark Gerstein[1], Flora M. Vaccarino[1]

[1]Program in Neurodevelopment and Regeneration, Yale University, New Haven, CT, United States, [2]Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA, United States

Reprogramming human somatic cells into induced pluripotent stem cells (iPSC) has been suspected of causing genomic instability and leading to generation of de novo genomic variants. To explore this issue, we performed a whole-genome and transcriptome analysis of 20 human iPSC lines derived from primary skin fibroblasts of 7 individuals using next-generation sequencing. We find that, on average, an iPSC line manifests two copy number variants (CNVs) not apparent in the fibroblasts from which the iPSC was derived. Using qPCR, PCR, and digital droplet PCR (ddPCR) to amplify across the CNVs' breakpoints, we show that at least 50% of those CNVs are present as low frequency somatic genomic variants in parental fibroblasts and are manifested in iPSC colonies due to their clonal origin. We observed similar effect for single nucleotide variants (SNVs). Hence, reprogramming per se does not induce a large amount of genomic variation, while the amount of somatic mosaicism in the tissue used to produce iPSCs could be a challenge and, at least, needs to be taken into account. We also carried out correlative analyses between the detected manifested CNVs and gene expression determined by RNA-Seq from the same iPSC lines.

When analyzing expression levels (by RPKM) of genes intersecting manifested CNVs in iPSCs we found a clear tendency (p-value of 0.01 by Fischer's exact test) of increase in expression for genes in duplications and decrease in expression for genes in deletions. However there were also genes in deletion CNVs with increased expression and genes in duplication CNVs with decreased expression, an observation that invites further analysis to probe the epigenetic regulation of the affected loci.

W-2184

AN IPS-DERIVED PLATFORM FOR THE STUDY OF MYOCARDIAL INFARCTION-ASSOCIATED DNA VARIANTS

Caroline Becker[1], Lauren Comisar[2], Eilene Duberow[1], Laurence Daheron[1], Vasan Ramachandran[3], Christopher J. O'Donnell[3], Kiran Musunuru[2], Chad A. Cowan[2]

[A]Harvard Stem Cell Institute, [B]Department of Stem Cell and Regenerative Biology, [1]Harvard University, Cambridge, MA, United States, [2]NHLBI Framingham Heart Study, Framingham, MA, United States

The discovery of new and effective treatments for human cardiovascular diseases requires the identification and validation of novel disease mechanisms. Recently, studies of genomic variation entered a new phase in which unbiased genome-wide association studies (GWAS) can identify novel genetic loci associated with common diseases. We have recently described 95 loci associated with blood lipid levels -- LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), or triglycerides -- which are strongly associated with risk for myocardial infarction (MI) in the NHLBI Framingham Heart Study (FHS) and other population cohorts. Much work will be needed to translate the novel associations into functional insights and, ultimately, therapies to reduce the risk of MI.

A key step is to determine how these genetic loci affect phenotypes in human tissue types relevant to lipid metabolism, principally liver and adipose. We have performed expression quantitative trait locus (eQTL) analyses of genotype vs. gene expression in surgical liver and adipose tissue samples from patients; from this work, we found a strong association between an LDL-C- and MI-associated SNP on chromosome 1p13 -- rs12740374 -- and hepatic expression of the *SORT1* gene. However, these studies were limited by scarcity of actual human tissue and the inability to address key cellular phenotypes such as lipoprotein secretion. Thus, there is a need to establish infinitely renewable sources of hepatocytes and adipocytes from patients of defined genotypes.

Here we describe our proposal to generate iPS cell lines from ~200 individuals in the Framingham Offspring Cohort with defined genotypes at chromosome 1p13 rs12740374. We have collected blood samples from over 2260 donors for reprogramming, and have generated 60 iPS lines from 13 donors. With respect to the MI-associated SNP, six donors were of the homozygous major genotype (that is associated with increased LDL-C and risk of MI), four were of the homozygous minor genotype, and three were of the heterozygous genotype. We plan to differentiate these iPS cells into functional hepatocytes and adipocytes and perform gene expression, metabolomic profiling and functional assays. Our goal is to identify new disease mechanisms and ultimately develop new, improved therapies to reduce the risk of MI.

W-2185

PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS RECAPITULATE HEMATOPOIETIC ABNORMALITIES OF JUVENILE MYELOMONOCYTIC LEUKEMIA

Prasuna Paluru[1], Shilpa Gandre-Babbe[2], Chiaka Aribeana[4], Stella T. Chou[2], Tasian K. Sarah[3], Silvia Bresolin[5], Lin Lu[1], Spencer K. Sullivan[2], Deborah L. French[1], Mignon L. Loh[6], Weiss J. Mitchell[2]

[A]Department of Pathology, [B]Division of Hematology, [C]Division of Oncology, [1]Childrens Hospital of Philadelphia, Philadelphia, PA, United States, [2]PennPREP Post-Baccalaureate Research Education Program, The University of Pennsylvania, Philadelphia, PA, United States, [3]Department of Women's and Children's Health, University of Padova, Padova, Italy, [4]Department of Pediatrics, Benioff Children's Hospital and the Helen Diller Comprehensive Cancer Cent, University of California, San Francisco, CA, United States

Juvenile Myelomonocytic Leukemia (JMML) is an aggressive childhood myeloproliferative disorder caused by NF1, NRAS, KRAS, PTPN11, or CBL mutations that induce Ras pathway activation with associated hypersensitivity to cytokine stimulation in myeloid progenitor cells. Understanding the pathophysiology of JMML and developing new treatments is constrained by limited access to relevant patient material. To address this problem, we generated induced pluripotent stem cell (iPSC) lines from malignant cells of two JMML patients with somatic heterozygous E76K missense mutations in PTPN11, which encodes the non-receptor tyrosine phosphatase SHP-2. In vitro differentiation of JMML iPSCs produced myeloid cells with increased proliferative capacity, hypersensitivity to granulocyte-macrophage colony-stimulating factor (GM-CSF), and sustained STAT5 and ERK phosphorylation, similar to what is observed in primary JMML cells from patients. Pharmacological inhibition of MEK kinase in iPSC-derived JMML cells inhibited basal activation of pERK, as well as abrogated GM-CSF-induced pERK, providing rationale for a potential targeted therapy. Our studies offer renewable sources of biologically relevant human cells in which to explore the pathophysiology and treatment of JMML. More generally, we illustrate the utility of iPSCs for in vitro modeling of a human malignancy.

W-2186

ERYTHROCYTE INDUCTION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS INDEPENDENT OF SOMATIC CELL ORIGIN

Isabel Dorn[1], Peter Schlenke[2], Martina Radstaak[3], Katharina Klich[1], Marcos J. Arauzo-Bravo[1], Simon Santourlidis[4], Boris Burr[1], Jeong Beom Kim[5], Martin Einhaus[6], Hans R. Schoeler[1], Holm Zaehres[1]
[1]Max Planck Institute for Molecular Biomedicine, Muenster, Germany, [2]Institute for Transfusion Medicine and Transplantation Immunology, University of Muenster, Muenster, Germany, [3]Max Planck institute for Molecular Biomedicine, Muenster, Germany, [4]ITZ, Heinrich-Heine-University, Düsseldorf, Germany, [5]Ulsan National Institute of Science and Technology, Ulsan, Korea, Republic of, [6]LADR, Geesthacht, Germany

Epigenetic memory in induced pluripotent stem (iPS) cells in
regard to their donor cell type of origin could lead to variations in their
differentiation capacities. We have generated iPS cells from human neural stem cells (NSC-iPS) and human cord blood derived CD34+ hematopoietic stem cells (HSC) (CD34-iPS) as well as fibroblasts (Fib-iPS) and evaluated their differentiation potential into hematopoietic precursor and mature red blood cells (RBC). Erythrocytes represent a defined end point of blood differentiation, while self-renewing, repopulating HSC, have not been successfully differentiated from human pluripotent stem cells until now. For hematopoietic induction, iPS cells were allowed to form embryoid bodies (EBs) under cytokine stimulation for 21 days. Thereafter, EBs were dissociated and single cells were applied to a three-step protocol for human erythropoiesis for additional 18-21 days. Hematopoietic differentiation was analysed by flow cytometry (CD43, CD34, CD45, CD36, glycophorin A) and colony formation in semisolid media. We have found a similar hematopoietic induction potential among our cell lines. After EB dissociation on day 21, hematopoietic commitment, measured by CD43 expression, was 31.9%±20.1% for CD34-iPS, 18.6%±10.3% for NSC-iPS and 17%±13% for Fib-iPS. Colony-forming unit assays demonstrate a similar distribution of myeloid (CFU-M/CFU-GM), erythroid (BFU-E/CFU-E) and more immature mixed (CFU-GEMM) colonies among iPS cell lines (CD34-iPS: 66±37 myeloid, 11±6 erythroid, 3±3 CFU-GEMM; NSC-iPS 77±52 myeloid, 3±2 erythroid, 1±1 CFU-GEMM; Fib-iPS: 58±28 myeloid, 6±7 erythroid, 1±1 CFU-GEMM). Hematopoietic cells further developed into erythroid precursors as determined by >90% expression of glycophorin A, followed by maturation into normoblasts and partially enucleated RBC (enucleation rate CD34-iPS 27.3%±4.9%, NSC-iPS 28.4%±10.9%, Fib-iPS 20.7%±5%). All human iPS derived mature erythrocytes predominantly present fetal hemoglobin (85%-90%), some embryonic (6%-7%) and only a minor amount of adult hemoglobin (2%-3%) as demonstrated by high performance liquid chromatographie. We have performed genome-wide DNA methylation profiling, giving some evidence of epigenetic marks during reprogramming. Our data indicate and support other data that epigenetic differences in human iPS cell lines generated of different donor cell type of origin (ectodermal neural versus mesodermal blood cells or fibroblasts) do not have a significant impact on their differentiation potential towards blood lineages.

W-2187

PURIFYING AND EXPENDING HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS FOR DERIVING INDUCED PLURIPOTENT STEM CELLS

Mu-Hua Chung[1], Chia-Nan Liao[2], Yi-Shiu Chen[1], Lung-Bin Lu[1], Akon Higuchi[3], Qing-Dong Ling[1]
[1]Cathay Medical Research Institute, Cathay General Hospital, New Taipei City, Taiwan, [A]Graduate Institute of Systems Biology and Bioinformatics, [B]Department of Chemical & Materials Engineering, [2]National Central University, Chungli, Taiwan

Induced pluripotent stem cells (iPSCs) have become a significant source for regenerative medicine and tissue engineering. Recent studies demonstrated that the techniques of iPSCs are able to be applied in clinical area such as personal medicine, tissue engineering and drug discovery. However, the somatic source from human for deriving human iPSCs is becoming an important issue. Thus developing an uncomplicated and efficient method to collect source cells is imperative in clinical application. In this study, we established a model to purify and expend mononuclear cells from human peripheral blood (PBMCs) with a simple and efficient procedure for inducing pluripotent stem cells. We isolated PBMCs from 3ml of whole blood collected from patients through by Ficoll gradient method and expanded cells with ALyS505N medium plus interleukin 2 (IL2) in tube for primary culture. Our results showed that a significant increase in cell numbers were up to above 600% in primary and second passages expending, as well as the types of cells were confirmed by flow cytometry. The human iPSCs were derived from cells in second passages by retroviral transduction of defined four transcription factors: Oct4, Sox2, Klf4 and c-Myc. However, expanded PBMCs were able to be continually culture to third passages in furthermore and the cells growth rate has decreased with the times of passage. These results indicate that our developed methods for isolating and expanding PBMCs could provide an efficacious process and desirable cells source for deriving the human iPSCs in clinical application in the future.

W-2188

GENE EXPRESSION SIGNATURE OF PORCINE IPS CELLS FROM DIFFERENT LABS SHOWS THE PROFILES DISSIMILAR TO MOUSE AND HUMAN

Yajun Liu, Huayan Wang
Dept. Biotechnology, Northwest A&F University, Yangling, Shaanxi, China

Some issues such as the incomplete reprogramming of porcine iPS cells(piPSCs), lacking the detailed database for porcine gene regulatory network and the difficulty to create the chimera animal of piPSCs precluded the application of this technology. To solve above problems and better understand the mechanism of porcine cell reprogramming, we analyzed the transcriptional profiles of porcine iPS cell lines using Affymetrix GeneChip Porcine Genome Array. Porcine iPS cell lines were
derived from our lab (30AC5 and PS24, which met all criteria required for a claim of pluripotency), Dr. Xiao's lab (iPF4-2, which was used to generate the cloned piglets), Dr. West's lab (piPS-w, which was used to create chimera pig) and Dr. Roberts' lab (IC1, ID4, ID6 and pESK, which data were extracted from GEO, GSE15472). Our results demonstrated that cell surface proteins of EpCAM (epithelial cells adhesion molecule) and Rex1 were significantly upregulated in complete reprogrammed porcine iPSCs, but not in partially reprogrammed cells, suggesting that these genes could be markers for evaluating porcine cell reprogramming. The number of upregulated genes in pig iPS cells was 1846 out of 23,937 gene probes. Ingenuity Pathways Analysis (IPA) revealed several pathways that might play important roles during the porcine cell reprogramming, such as Sertoli-Sertoli Cell Junction signal pathway, Ga12/13 signal pathway, and BMP signal pathway etc. We analyzed gene expression level of the six key developmental signaling pathways, including JAK-STAT, NOTCH, TGFb, WNT, MAPK and VEGF. These results demonstrate that the core transcriptional network to maintain pluripotency and self-renewal in pig may be different from mouse and human. Pig iPSCs are lack specification naïve state markers, such as Kruppel-like factors (Klf2/4) and Tbx3, whereas, the markers for primed state (e.g. Otx2 and Fabp7) were upregulated significantly. The similarities among

piPSCs, hiPSCs and mEpiSCs underscored the possibility that traditional culture conditions cannot used to maintain long-term proliferation of pig pluripotent cells with inherent instability of naïve pluripotency. We also identified that aberrant silencing of Dlk1-Dio3 domain was instantaneous response for factor-induced reprogramming in different species. Aberrant silencing of Dlk1-Dio3 domain is a common outcome upon induced reprogramming in pig, mouse and human. Real time PCR results confirm the notion that silencing of this cluster is a common outcome upon factor-mediated reprogramming in pig. The defect in piPS partially explains the rare success of the birth of chimeric and cloned offspring. In conclusion, our results provide a fundamental resource for better understanding of the complex genetic network that maintains pluripotency in pig.

W-2191

SCREENING OF NEW FACTORS THAT INFLUENCE THE PROCESS OF SOMATIC REPROGRAMMING MEDIATED VIA FORCED EXPRESSION OF SPECIFIC GENE

Tatsuya Yamakawa[1], Yoshiko Sato[1], Yuki Takase[1], Naoki Goshima[2], Keisuke Okita[1], Shinya Yamanaka[1]

[1]Department of Reprogramming Science, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [2]Biomedicinal Information Research Centre, National Institute of Advanced Industrial Science and Technology, Tokyo, Japan

Induced pluripotent stem (iPS) cells were initially generated from mouse and human fibroblast by retrovirus- or lentivirus- mediated infection of following four factors, Oct3/4, Sox2 with either Klf4 and c-Myc or Nanog and Lin28. Human iPS cell could be applicable to clinical application such as disease modeling, drug discovery and toxicology. However, it is still unclear what happens during the somatic reprogramming via forced expression of several genes. Until now, many researchers have found out the new reprogramming genes that can improve reprogramming efficiency or replace the first-reported 4 genes. Some of these genes have been reveled to have direct impact on reprogramming process without affecting cell proliferation. The analysis of such genes may be useful to clear mechanisms of cellular reprogramming, which is important not only for stem cell biology but also for clinical application of iPS cell.

To find new genes that influence on the reprogramming process, we established a screening system. As candidates, we employed human cDNA library consisted of 2500 transcription factors and 300 kinases. Each candidate genes were transfected into human dermal fibroblast with basic 5 factors (OCT3/4, SOX2, KLf4, L-MYC and LIN28) via plasmid method and evaluated whether they influenced on iPS generation efficiency as the result of their impact on reprogramming process. We used mock as a negative control and GLIS1 as a positive control. Induction efficiency of each sample was calculated by comparison between the number of human ES-like colonies induced by the each factor and mock.

This kind of screening has usually been performed by using mouse embryonic fibroblast and virus-mediated transfection method, because of their simple and easy procedure to handle many samples. In contrast, we employed human fibroblast as the cell source and plasmid-mediated transfection for transgene expression. The reason why we chose these methods is that the reprogramming factors don't always work in the same way as they were originally reported when used in different methods or species. Our screening system will enable to find new factors which could provide some insights of reprogramming process from human cells, especially in the methods enable to medical application. Still the screening is going on, we show the development of our screening assay using automatic colony-count system with BioStationCT and summary of the results. We detected several factors which positively or negatively affected on the induction efficiency. These library-derived new factors can also influence iPS induction from fibroblasts of two different donors. Interestingly, some of these positive factors have the same protein motif, suggesting they have some important function during somatic reprogramming. Additionally, we checked the mRNA expression changes of both positive and negative factors during iPS generation. By analyzing factors which affect on iPS induction efficiency, we are trying to uncover underlying mechanisms of somatic reprogramming. The new factors we found would be also useful to develop appropriate reprogramming methods for clinical application.

W-2192

BENEFICIAL EFFECTS OF METABOLIC CONTROL IN THE ACQUISITION AND MAINTENANCE OF PLURIPOTENCY

Myung Jin Son[1], Mi-Young Son[1], Binna Seol[1], Min-Jeong Kim[1], Chae Hwa Yoo[1], Hee–Jun Na[1], Myung-Kwan Han[2], Yee Sook Cho[1]

[1]Regenerative Medicine Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Korea, Republic of, [2]Department of Microbiology, Institute for Medical Sciences, Chonbuk National University Medical School, Jeonju, Korea, Republic of

Cross-talk between intracellular signaling pathways has been extensively studied to understand the pluripotency of human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs); however, the contribution of NAD^+-dependent pathways remains largely unknown. Here, we show that NAD^+ depletion by FK866 (a potent inhibitor of NAD^+ biosynthesis) was fatal in hPSCs, particularly when deriving pluripotent cells from somatic cells and maintaining pluripotency. NAD and its precursors (nicotinamide (NAM) and nicotinic acid (NA)) fully replenished the NAD^+ depletion by FK866 in hPSCs. However, only NAM effectively enhanced the reprogramming efficiency and kinetics of hiPSC generation and was also significantly advantageous for the maintenance of undifferentiated hPSCs. Our molecular and functional studies reveal that NAM lowers the barriers to reprogramming by accelerating cell proliferation and protecting cells from apoptosis and senescence. Our findings establish that adequate intracellular NAD^+ content is crucial for pluripotency; the distinct effects of NAM on pluripotency may be dependent not only on its metabolic advantage as a NAD^+ precursor but also on the ability of NAM to enhance resistance to cellular stress.

W-2192

A NOVEL UTF1-TOMATO PLURIPOTENCY REPORTER MOUSE

Peter Droge[1], Amir Morschedi[1], Wei-Ping Yu[2]

[1]Nanyang Technological Uiversity, Singapore, Singapore, [2]Biomedical Sciences Institute, Singapore, Singapore

The Utf1 protein (undifferentiated embryonic cell transcription factor 1) was first described in 1998 by Okuda *et al.* in an Article published in the *EMBO J.* Today, Utf1 is established as a member of the core transcription network which defines and regulates the pluripotent embryonic stem cell state. Our previous studies have shown that the human *Utf1* control elements (i.e. the promoter and the 3`-enhancer) can be employed as very reliable pluripotency reporter in human and mouse ES/iPSCs. In agreement with this, a more recent study by R. Jaenisch and coworkers published in *Cell* identified *Utf1* transcription, which is a direct downstream target gene of Oct4 and Sox2 (and presumably Nanog), as one of the most reliable predictors for the execution of the complete reprogramming program during iPSC formation. Furthermore, it was shown in a separate recent *Cell* study that the Utf1 protein ensures rapid proliferation and coordinated differentiation of ESCs through chromatin modification and the control of mRNA decapping. Hence, it appears timely to briefly present here our preliminary assessment of a novel *Utf1-tomato* knock-in pluripotency reporter mouse. With further characterization, this animal system has the potential of becoming a very useful tool for a number of *ex vivo* and *in vivo* studies, possibly also permitting questions beyond the topic of iPSC formation.

W-2194

GENERATION OF INDUCED PLURIPOTENT STEM CELLS USING RAT MESENCHYMAL STROMAL CELLS ORIGINATED FROM A NEW MODEL OF METABOLIC SYNDROME

Nana Ninagawa Takenaka[1], Mami Kobayashi[1], Yuka Kawabata[1], Nagata Kohzo[2], Shigeko Torihashi[1]

[A]Department of Rehabilitation Sciences, [B]Department of Pathophysiological Laboratory Sciences, [1]Nagoya University Graduate School of Medicine, Nagoya, Japan

We recently established a new animal model of metabolic syndrome (MetS), the DahlS.Z-*Lepr*^{fa}/*Lepr*^{fa} (DS/obese) rat, by crossing Dahl salt-sensitive (DS) rats with Zucker rats harboring a missense mutation in the leptin receptor gene (*Lepr*^{fa}). Body weight and visceral. subcutaneous fat masses were significantly increased, and blood pressure was higher in DS/obese rats when compared with homozygous lean littermates (DS/lean) rats (Hattori et al. 2011). Although the phenotype of DS/obese rats is similar to that of MetS in humans, the pathophysiological and metabolic characteristics in each cell type remain to be clarified. Hence, induced pluripotent stem cells (iPSCs) derived from MetS rats serve to promote investigations *in vitro*.
Here, we report the establishment of the rat iPS cells (riPSCs) from DS/obese and DS/lean rats to investigate cell biology of MetS *in vitro*. We generated riPSCs from mesenchymal stromal cells (MSCs) collected from adult rat subcutaneous adipose tissues of DS/obese and DS/lean rats individually. MSCs from both rats were introduced three mouse reprogramming factors (*OCT3/4, SOX2, and KLF4*) by lentiviral vectors according to the previous report with some modifications (Kobayashi et al. 2009). They were then cultured with SNL feeder cells in the presence of exogenous leukemia inhibitory factor (LIF) to establish iPS cells as o-riPSCs and l-riPSCs, respectively. The lentiviral transduction yield ES cell-like colonies from individual rat MSCs, and these colonies with EGFP were alkaline phosphatase-positive, indicating pluripotent stem cells. Then, we tried to pick up ES cell-like colonies, trypsinized them into single cells, and transferred them to 24-well plates for further expansion. Established riPSCs could expand without any morphological changes. Like mouse ES cells, both o-riPSCs and l-riPSCs expressed stage-specific embryonic antigen (SSEA)-1, and Nanog protein. We also confirmed that both riPSCs expressed many undifferentiated ESC-marker genes, including *Nanog, OCT3/4, Klf4, Rex1, Tdgf2, Sox2, Eras,* and *fibroblast growth factor 4 (Fgf4)* by RT-PCR. We examined the capacity of riPSCs for *in vivo* differentiation, and assessed teratoma formation with microscopy of hematoxylin/eosin (HE) stained sections. The results suggest that our riPSCs had been reprogrammed into pluripotent state and possessed the capacity for differentiation into cells of three germ layers.
Thus, we succeeded in generating riPSCs from MSCs of DS/obese and DS/lean rats using only three reprogramming factors. These riPSCs will serve as highly effective tools for the investigation of MetS pathophysiology *in vitro*.

W-2195

DERIVATION AND CHARACTERIZATION OF RAT IPS CELLS USING A MOUSE STEMCCA REPROGRAMMING VECTOR

Pavan Rajanahalli, Mark L. Weiss
Kansas State University, Manhattan, KS, United States

Rats are considered an important animal model system by having many similarities to humans in terms of physiological processes of the cardiovascular, metabolic and neurological systems. Induced pluripotent stem cell (iPSC) technology has great potential to advance in the field of regenerative medicine by generating patient-specific stem cell for treatment or in vitro disease modeling. Here, we derived rat iPSC's from Fischer 344 embryonic fibroblasts using a mouse STEMCCA cre-excisable reprogramming vector expressing Oct-4, Klf4, Sox-2 and c-Myc. Once rat ESC-like colonies were derived, we removed the transgene vector and demonstrated that one of the clones tested showed a normal karyotype by G-banding. The iPSC colonies demonstrate a morphology that is similar to rat embryonic stem cells (ESCs), are positively stained for pluripotency markers such as alkaline phosphatase, Oct-4, Nanog, Sox-2 and SSEA-1. The rat iPSCs have been maintained more than 27 passages. Using a custom-designed microarray, we compared the gene expression profile of rat iPSCs to rat ESCs and there was good similarity between the two with the exception of Cdx2 expression which is expressed in rat ESCs. We plan to further characterize the rat iPSCs to determine whether they can undergo neural differentiation in vitro and whether the micro RNA expression of rat iPSCs is similar to rat ESCs.

W-2196

QUANTITATIVE FLUORESCENT IN VITRO ASSAY FOR MONITORING DIFFERENTIATION OF PLURIPOTENT STEM CELLS TOWARDS MYELINATING OLIGODENDROCYTES

Antje Arnold, Agatha Lyczek, Jiangyang Zhang, Miroslaw Janowski, Jeff WM Bulte, Piotr Walczak

Dept. of Radiology, The Johns Hopkins University School of Medicine, Baltimore, MD, United States

Evidence is mounting that transplantation of glial-restricted progenitor cells (GRPs) is a promising approach for restoration of brain function in patients suffering from de/dysmyelinating neurological disorders. Rodent studies demonstrated that transplantation of GRPs into the CNS results in the generation of new myelinating oligodendrocytes and robust therapeutic effect. It is imperative to develop techniques for non-invasive monitoring of graft functionality for clinical translation. To this end, we applied diffusion tensor MRI (DTI) for *in vivo* imaging of myelination in *shi/shi rag2*$^{-/-}$ mice transplanted with GRPs. *In vivo* DTI showed improvement of imaging parameters with reduced radial diffusivity within white matter structures in transplanted compared to control mice. Immunohistochemistry revealed robust myelination by transplanted cells and migration from ventricles to the brain parenchyma, particularly into white matter. This was in good agreement with MRI findings. The life span of transplanted *shi/shi rag2*$^{-/-}$ mice was extended. Our results strongly support this strategy with transplantation of GRPs for de/dysmyelination.

One limitation of this strategy is the inadequate access to human fetal tissue for the derivation of human GRPs. To overcome this issue, we propose to derive GRPs from pluripotent cells as an unlimited cell source. Dr. Goldman's group showed that derivation of GRPs from iPSCs requires an extremely lengthy differentiation procedure (120 days) exemplifying another challenge. To address this, we have developed a robust *in vitro* differentiation assay using fluorescence as a readout for oligodendrocyte differentiation with the goal of accelerating the differentiation process.

Here, we generated iPSCs derived from proteolipid protein-green fluorescent protein (PLP-GFP) transgenic mice. PLP-GFP iPSCs did not express GFP as GFP expression is induced under the oligodendrocyte-specific PLP promoter. We are differentiating iPSCs towards GRPs and mature oligodendrocytes, with the GFP expression used as the primary quantitative readout for assessing efficiency of this differentiation process. To properly model and stimulate *in vitro* differentiation towards myelinating oligodendrocytes, we applied GRPs and neural stem cells (NSCs) to a co-culture system. NSCs were derived from transgenic mice expressing DsRed under β-actin promoter. With this combination of DsRed-NSC and inducible PLP-GFP iPS-derived GRPs we are able to monitor the differentiation of iPSCs towards mature oligodendrocytes with fluorescence. Preliminary data on oligodendrocyte differentiation of primary PLP-GFP+ GRPs demonstrated that GFP fluorescence intensity increased during the differentiation process. Oligodendrocytes were evaluated based on the morphology and immunostaining for oligodendrocyte-specific markers (Olig2, MBP). We are currently applying the same differentiation protocol for our PLP-GFP+ GRP-iPSCs with efficiency of differentiation assessed by longitudinal measurements of GFP expression, immunostaining and qPCR for oligo-, neuronal- and *astrocyte-specific* markers (Olig2, MBP, Tju1, GFAP). Further characterization will include transplantation into neonatal *shi/shi rag2*$^{-/-}$ mice with DTI. In conclusion, we present an *in vitro* approach for monitoring differentiation of iPS cells towards myelinating oligodendrocytes using fluorescence readout and thus facilitating optimization of differentiation protocol.

W-2197

A ROBUST SECONDARY SYSTEM FOR HIGH-THROUGHPUT STUDIES OF REPROGRAMMING AND PLURIPOTENCY PATHWAYS IN HUMAN CELLS

Davide Cacchiarelli[1], Magali Soumillon[1], Cole Trapnell[1], Xiaolan Zhang[2], Casey Gifford[1], Veronika Akopian[1], Alexander Meissner[1], John L. Rinn[1], David T. Scadden[1], Eric S. Lander[2], Tarjei S. Mikkelsen[1]

[1]Harvard Stem Cell Institute and Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States, [2]Broad Institute of Harvard and MIT, Cambridge, MA, United States

Reprogramming of somatic cells to induced pluripotent stem cells (iPSC) is an inefficient and long process. Reprogramming efficiency is highly dependent on the stoichiometry of the ectopically expressed reprogramming factors, which is difficult to obtain in a uniform manner with many delivery systems. Drug-inducible secondary systems, consisting of fibroblasts differentiated from iPSCs obtained through doxycycline-inducible reprogramming factors, represent genetically homogeneous systems that partially address this technical issue. Unfortunately, existing sec-

ondary systems suffer from several limitations, mainly related to the limited lifespan and expansion capability of the secondary somatic cells. Senescence affects the consistency of both derivation and reprogramming of secondary fibroblasts, making the system challenging to use for high-throughput screening and genome editing-based studies.
To generate an improved human secondary system, we first derived new iPSC lines using a doxycycline-inducible polycistronic Oct4-Klf4-Myc-Sox2 vector to minimize integration events and to maintain a controlled stoichiometry. Secondary fibroblast clones obtained from directed differentiation of these inducible iPSCs were then engineered to extend their life span. This process, coupled with optimization of defined growth conditions, facilitated the generation of a secondary cell line that is capable of a undergoing a large number of cell divisions without losing its high reprogramming potential or the genetic and epigenetic characteristics of primary cells.
iPSCs derived from the new secondary cells are directly comparable to those obtained from established primary reprogramming strategies, with the added advantage that the reprogramming is highly consistent and uniform. We have applied this unique feature to extensively characterize the molecular events occurring during reprogramming of human cells by isolating different intermediate populations and profiling them using high-throughput strategies, including RNA-seq and ChIP-seq. In addition, we are testing the robustness of many assays in this system for the study of reprogramming and pluripotency pathways, including high-throughput functional RNAi screening, massively parallel reporter assays (MPRA) and synthetic nuclease-mediated genome editing..
This newly developed secondary system represents a handy and powerful tool for testing functional hypotheses and has the potential to allow deep dissection of the molecular events underlying cellular reprogramming in human cells.

W-2201

RNA SPLICING REGULATION DURING SOMATIC CELL REPROGRAMMING

Sho Ohta[1], Eisuke Nishida[1], Shinya Yamanaka[2], Takuya Yamamoto[2]
[A]Graduate School of Biostudies, [B]CiRA, iCeMS, [1]Kyoto Univ, Kyoto, Japan

Despite the extensive studies on somatic cell reprogramming, the molecular mechanisms underlying the reprogramming process are still unclear. Here we report our analysis on changes in alternative splicing during reprogramming. By combining deep sequencing with high-throughput quantitative RT-PCR, we revealed drastic changes in genome-wide splicing profiles during the reprogramming process. Our analysis identified more than 600 genes, whose splicing patterns are changed during iPS cell induction from mouse embryonic fibroblasts (MEFs). Computational analysis of primary sequences demonstrated that several motifs are enriched in sequences within, and adjacent to, the differentially expressed skipped exons between MEFs and iPS cells. Moreover, we also found that the lengths of the introns around exons, which are preferentially included in iPS cells, tend to be shorter. In addition, we performed siRNA screen for the RNA binding proteins that are responsible for the splicing events in pluripotent stem cells. Consequently, we obtained a number of RNA-binding protein-encoding genes whose depletion causes alteration in splicing patterns in pluripotent stem cells, and some of which are necessary for somatic cell reprogramming. These findings indicate that post-transcriptional as well as transcriptional regulation mechanisms are integral parts of the molecular network in the reprogramming process and the pluripotency maintenance. Our results should contribute to elucidation of the mechanisms underlying somatic cell reprogramming.

W-2202

INVESTIGATION OF THE X CHROMOSOME VIA USE OF PLURIPOTENT STEM CELLS WITH HUMAN X ANEUPLOIDIES

Antonia A. Dominguez, Dian Yang, Shawn Chavez, Rosaria Chiang, Renee Reijo Pera
Stanford University, Stanford, CA, United States

Our goal is to examine the role of the human X chromosome in germ line and somatic development in an induced pluripotent stem cell (iPSC) system. For this purpose, we are developing and characterizing iPSC lines from women with different X chromosome dosages. Turner Syndrome (TS) females have partial or complete loss of the second

X chromosome, resulting in a 45,X karyotype. Three percent of pregnancies begin as 45,X; however, 99% of these are spontaneously terminated, and females who live have a wide variety of clinical characteristics including short stature and infertility. Triple X syndrome (XXX) is a trisomy of the X chromosome and results in tall stature, infertility and sporadic learning disorders. Here, we report derivation of iPSCs from four TS females and two control females. All iPSCs maintained original karyotypes, expressed key pluripotency markers and spontaneously differentiated in vivo and in vitro into the three germ layers. We expect that iPSCs derived from TS and XXX females may accurately replicate the haploinsufficiency and X chromosome aberrations observed in the original patient, making them an ideal model to investigate the role of the X chromosome in early development and germ cell development and test this concept further.

First, we determined that expression levels of X chromosome genes were similar between single blastomeres of day 2 and day 3 human embryos and iPSCs, which we used to model early development. We then investigated X chromosome gene expression differences amongst TS, control and XXX iPSC lines. We analyzed the expression of 96 genes including X-linked and pluripotency genes, at the single cell level and observed small but statistically insignificant differences in gene expression. We also compared the whole transcriptome across different iPSCs lines via RNA-seq and observed differential expression level of multiple X chromosome genes was discovered. We are currently examining differences in more detail.

Infertility in females with only one X chromosome has been previously established, however, no specific X-linked genes have been correlated with infertility because of the lack of phenotype in the TS mouse model (39,X). To address whether the absence or gain of the second X chromosome results in defects in the formation of primordial germ cells (PGCs) we are currently carrying out directed differentiation to the PGC lineage using BMPs, retinoic acid and the VASA:GFP reporter construct. Single cell expression analysis as well as RNA-sequencing is being conducted to analyze the expression of X-linked and germ cell genes as TS, control and XXX hiPSC lines as they are differentiated into the germ cell lineage.

Overall, despite the large variety of clinical characteristics seen in TS and XXX females, only one gene has been linked to specific phenotype in TS females. It is hoped that results of our studies will identify expression differences of X-linked genes that can be investigated further for their necessity in normal early development and proper germ cell development.

Reprogramming

W-2203

CHARACTERIZATION OF AID-DEFICIENT IPS CELLS

Ren Shimamoto[1], Naoki Amano[1], Tomoko Ichisaka[1], Akira Watanabe[1], Keisuke Okita[1], Shinya Yamanaka[2]

[1]Center for iPS Cell Research and Application (CiRA), Kyoto, Japan, [2]Center for iPS Cell Research and Application (CiRA), Yamanaka iPS Cell Special Project, Gladstone Institute of Cardiovascular Disease, Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto, Japan

IPS cells can be generated by introducing defined factors into somatic cells. It has been shown that epigenetic status, such as DNA methylation and histone modification, changes dramatically during iPS cells generation. For instance, DNA methylation level of *Oct4* and *Nanog* promoters decrease during iPS cell generation. However, the underlying mechanism of demethylation is still unclear.

Activation-induced cytidine deaminase (Aid) converts methylated and unmethylated cytosine to thymine and uracil respectively by removing amine. Recently some papers suggested that Aid is involved in DNA demethylation during developmental processes in zebrafish and mouse. Interestingly, AID was also shown to be involved in DNA demethylation activity in cell-fusion-mediated reprogramming of mammalian cells. One recent report revealed that the transient knockdown of Aid hampered the initiation of mouse iPS cell generation. Taken together, we hypothesized that Aid would be involved in DNA demethylation during iPS cell generation.

In this study, we employed loss of function approach and examined the effect of Aid depletion on DNA methylation status in iPS cells. Aid knockout mice were crossed with Nanog-GFP mice which have GFP reporter under the control of a pluripotent stem cell marker, Nanog. Oct3/4, Sox2, Klf4 and c-Myc were introduced into Aid knockout mouse

embryonic fibroblasts by retrovirus. Contrary to our expectation, Nanog-GFP positive iPS colonies emerged from Aid KO MEFs. The induction efficiency was not different from that of wild-type iPS cells (Wt iPS cells). In addition, the overexpression of Aid did not affect the induction efficiency.
We examined morphology, proliferation, differentiation potential, RNA expression and DNA methylation of Aid KO iPS cells. Aid KO iPS cells showed normal proliferation and gave rise to three germ layers in teratoma forming assay. Moreover, Aid KO iPS cells could give rise to chimera by blastocyst injection. Microarray analysis showed that there were few differences between Aid KO iPS cells and Wt iPS cells. These results suggest that Aid KO iPS cells were similar to Wt iPS cells in terms of ability of self-renewal and pluripotency.
In the DNA methylation analysis, first *Nanog* and *Oct3/4* promoter regions were analyzed. However, in Aid KO iPS cells both regions were hypomethylated similar to that of Wt iPS cells. The global DNA methylation level examined by LINE1 and B1 repeat sequences did not show significant difference. Then, we searched for specifically methylated regions in Aid KO iPS cells by comprehensive DNA methylation assay. The genomic fragments were concentrated by immunoprecipitation using methylted CpG binding protein and were analyzed by deep sequencing. However, only few differences were found between Wt and Aid KO iPS cells. Consequently, the effect of Aid depletion on DNA demethylation was not observed in our analyses.

W-2204

GLOBAL ALLELIC EXPRESSION PROFILING OF INDUCED PLURIPOTENT STEM CELLS AND THEIR DIFFERENTIATED NEURAL STEM CELL PROGENY

Aaron R. Jeffries[1], Dafe A. Uwanogho[1], Graham Cocks[1], Leo W. Perfect[1], Emma L. Dempster[2], Jonathan Mill[2], Jack Price[1]

[1]Neuroscience, King's College London, London, United Kingdom, [2]Epigenetics Group, University of Exeter Medical School, Exeter, United Kingdom

Human stem cell populations show clonal heterogeneity. Such phenotypic diversity has implications for development, and for the use of stem cells in regenerative medicine. We have previously demonstrated random or stochastic choice of allelic expression occurring in a subset of non-imprinted autosomal genes in genetically identical human neural stem cell clones derived from fetal brain. DNA methylation and a specific pattern of histone modifications are both associated with allelic choice. In this study, we asked what happens to stochastic allelic choice if the neural stem cell clones were epigenetically reprogrammed and neuralized? We carried out global allelic expression profiling of human induced pluripotent stem cells (hiPSC) derived from a previously characterised clonal neural stem donor. We also neuralized these cells and isolated neural stem cell clones. Results: We find that while imprinted genes remain imprinted, autosomal genes originally undergoing random or stochastic choice of allelic expression in the donor neural stem cells switch to biallelic expression in the epigenetically reprogrammed hiPSC state. Subsequent neuralization of the hiPSCs and clonal isolation of the neural stem cells show at least 0.75% of genes undergoing stochastic choice of allelic expression. The majority of these genes represent new loci when compared to the original donor neural stem cell, but functional annotation of these new genes show a strikingly similar profile to that seen in the original neural stem cell study, namely overrepresentation of membrane bound or cell surface genes, a number of which are of neurodevelopmental in function. We conclude that the diversity of neural stem cells generated by stochastic monoallelic expression is lost when the cells are reprogrammed, but re-established in a stochastic fashion when the reprogrammed cells are neuralized.

W-2205

MOLECULAR MECHANISMS OF ASTROGLIA-TO-NEURON CONVERSION BY FORCED EXPRESSION OF PRONEURAL GENES

Giacomo Masserdotti[1], Sebastien Gillotin[3], Martin Irmler[2], Johannes Beckers[2], Francois Guillemot[3], Benedikt Berninger[4], Magdalena Goetz[1]

[A]Stem Cell Institute, [B]Institute for Experimental Genetics, [1]Helmholtz Zentrum, Munich, Germany, [2]Division of Molecular Neurobiology, National institution for Medical Research, London, United Kingdom, [3]Institute of Physiological Chemistry, University Medical Center Johannes Gutenberg University Mainz, Mainz, Germany

Direct conversion of differentiated cells into neurons is emerging as a promising alternative for cell-based therapies of neurodegenerative diseases, because it does not give rise to pluripotent intermediates. Yet little is known about the molecular mechanisms that induce direct neuronal reprogramming, thus limiting the development of more precise strategies aimed at obtaining pure, homogenous and specific neuronal subtypes.
To better understand the mechanisms underlying direct reprogramming, we employed astroglial cells as model system: in fact, they are distributed throughout the mammalian brain, thus representing a potential endogenous source of reprogrammable cells[1], and can be efficiently converted into functional neurons upon over-expression of two proneural genes, *Neurog2* and *Ascl1*[2,3] .
Astroglia cultures obtained from postnatal mouse cerebral cortex were transduced with retroviruses encoding an inducible system in which the cDNA of the proneural gene (either *Neurog2* or *Ascl1*) is fused to a modified estrogen receptor-binding domain (ERT2). Hence, the resultant protein is expressed but remains inactive unless cells are treated with tamoxifen, upon which inhibition is released. This allows for a temporally defined onset of the proneural gene's transcriptional activity. Indeed, *Neurog2*- and *Ascl1-ERT2*-transduced astrocytes generated neurons upon tamoxifen treatment, while no neurons were observed in control-treated *Neurog2*- or *Ascl1-ERT2*-transduced cells, thus providing an experimental system for further molecular analyses.
Unbiased transcription profile was performed on RNA extracted from astroglial cultures transduced with control viruses or *Neurog2*-(or *Ascl1*)-*ERT2* expressing viruses and treated with tamoxifen for 24 hours: interestingly, there is only a partial overlap between the genes induced by both Neurog2ERT and Ascl1ERT2, suggesting that the two proneural genes activate a different neurogenic program already at a very early stage of reprogramming, likely reflecting their capacity to direct astroglia towards distinct neuronal subtypes. On the other side, transcription factors downstream of both transcription factors are likely to contribute to the execution of a generic program of neurogenesis.
Indeed, loss-of-function experiments utilizing microRNAs against shared targets demonstrated their functional requirement for Neurog2-induced astroglia-to-neuron conversion; notably, these targets were not sufficient to reprogram cells on their own, but the combined expression could induce neuronal generation from postnatal astroglial cells, suggesting that each of them activates distinct and non-redundant branches of the Neurog2-induced program. These data provide a first insight into the early stages of direct reprogramming towards neurogenesis, highlighting the early activation of a neurogenic program, and identifying key factors required for the neuronal generation from postnatal astroglial cells.
This project was funded by Wellcome Trust and by the German Research Foundation (Deutsche Forschungsgemeinschaft).

.

1. S. Robel, B. Berninger, M. Gotz, *Nat Rev Neurosci* **12**, 88 (Feb, 2011)

W-2206

EFFECTS OF SUBSTRATE TOPOGRAPHY ON THE FUNCTIONAL NEURONS PRODUCED BY DIRECT REPROGRAMMING OF FIBROBLASTS

Karina Kulangara[1], Andrew F. Adler[1], Hong Wang[2], Malathi Chellappan[1], Ellen Hammett[1], Ryohei Yasuda[2], Kam W. Leong[1]
[A]Biomedical Engineering, [B]Neurobiology, [1]Duke University, Durham, NC, United States

Cellular reprogramming holds tremendous potential for cell therapy and regenerative medicine. Recently fibroblasts have been converted into induced neurons (iN) by overexpressing the transcription factors *Ascl1*, *Brn2* and *Myt1L.* This presents an attractive cell source for combating neurodegenerative diseases. *In vivo* the microenvironment presents physical and biochemical cues that influence cellular behavior. It regulates cellular function ranging

from adhesion and migration to proliferation. Here we investigate how substrate topography influences reprogramming of fibroblasts to induced neurons. Polystyrene substrates imprinted with microgratings or circular posts were used with non-patterned polystyrene samples as controls. We observed a significant reduction of the number of neurites per soma on the 5 [[Unsupported Character - Symbol Font ]]m gratings topography compared to the smooth control. The corresponding average length of the neurites was 296.6 [[Unsupported Character - Symbol Font ]]m versus 174 [[Unsupported Character - Symbol Font ]]m. When comparing mRNA expression of cells on smooth substrates to 5 [[Unsupported Character - Symbol Font ]]m gratings we detected 81 differentially expressed genes, including ones implicated in neuronal differentiation and cell projection organization like artemin, netrin, slit3, Thy1, growth arrest specific protein 1, and sphingosine-1-phosphate receptor 1. As characterized by electrophysiology, the induced neurons obtained on 5 [[Unsupported Character - Symbol Font ]]m gratings and smooth substrates were functional. The average membrane potential of iNs on smooth substrates was similar to iNs on 5 [[Unsupported Character - Symbol Font ]]m substrates; with an average resting membrane potential of -76 mV for iNs on smooth and -82 mV for iNs on 5 [[Unsupported Character - Symbol Font ]]m gratings. This study advocates a role of cell-topography interactions in shaping iN identity. It also suggests the potential of applying topographical cues to optimize the microenvironment for various direct reprogramming processes.

W-2207

BIOPHYSICAL REGULATION OF EPIGENETIC STATE AND CELL REPROGRAMMING

Timothy L. Downing[1], Jennifer Soto[1], Constant Morez[3], Timothee Houssin[4], Falei Yuan[1], Julia Chu[1], Ashley Fritz[2], Shyam Patel[1], David V. Schaffer[2], Song Li[1]

[A]Bioengineering, [B]Chemical Engineering, [1]University of California, Berkeley, Berkeley, CA, United States, [2]Biomedical Engineering, Ecole Polytechnique, Palaiseau, France, [3]University Lille Nord de France, F-59000, Lille, France

Cell reprogramming represents a major advancement in cell biology and has wide applications in regenerative medicine, disease modeling, and drug screening. Induced pluripotent stem cells can be produced from adult fibroblasts with the use of transcription factors and chemical compounds; however, the role of biophysical factors in cell reprogramming is not known. Here we show, for the first time, that biophysical cues, in the form of parallel microgrooves on the surface of cell-adhesive substrates, can significantly improve reprogramming efficiency and replace the effects of small molecule epigenetic modifiers. The mechanism behind this biophysical enhancement of cell reprogramming relies on mechanomodulation of epigenetic state -specifically, increases in histone H3 acetylation (AcH3) and histone H3 trimethylation (H3k4me3) on microgroove surfaces. Further investigation reveals that microtopography dramatically downregulates histone deacytylase (HDAC) activity and increases the expression of WD repeat domain 5 (Wdr5) protein -a subunit of H3 methyltransferase. Furthermore, microtopography promotes the upregulation of key epithelial-related genes, suggesting the initiation of a mesenchymal-to-epithelial transition, a required step in cell reprogramming. Disruption of actin-myosin contractility abolishes microtopographical regulation of epigenetics and cell reprogramming, confirming that the actin cytoskeleton is required for this epigenetic mechanomodulation. In addition, adult fibroblasts show a similar response on aligned nanofiber surfaces in cell reprogramming and histone modifications, providing additional evidence that various topographical cues can regulate cell reprogramming. Furthermore, micropatterning cells on islands of matrix proteins for tight regulation of cell shape demonstrates the critical role that cell deformation plays in actively changing epigenetic state. In summary, this study unravels how the microfeatures of cell-adhesive substrates can induce epigenetic modifications, which in turn lead to enhanced cell reprogramming. We believe this novel biophysical regulation of epigenetics and cell reprogramming has important implications in a number of complex biological processes and in the broad scope of cell biology.

W-2208

GENETIC ENGINEERING OF PIG PLURIPOTENT CELLS USING A NOVEL GENE TARGETING TECHNIQUE.

JONGPIL KIM, YaeJu Sung, BakSoon Bong
Biomedical Engineering, Dongguk University, Seoul, Korea, Republic of

Several genetically modified pig models have been produced by combining conventional gene targeting methods with somatic cell nuclear transfer. However, these conventional techniques are inefficient in mammalian somatic cells and provide little control over the site specificity and rate of exogenous DNAintegration. Recent studies showed that genetic engineering of cells with TAL Effector nucleases (TALEN) is rapidly gaining popularity as a means to enhance the rate and specificity of DNA modifications in plant and animal cells. Here we examine the genetic modification in pigs by combining TALEN technology with induced pluripoetent stem cells. These studies will open the door to genome targeting with a precision that was not previously possible in a large animal model and provide important considerations for their safe and effective use in modification of the swine genome, and future innovative applications of this technology in pigs will be discussed.

W-2211

PROGRAMMING THE HAIR FOLLICLE STEM CELL NICHE IN UNCOMMITTED SKIN FIBROBLASTS

Carlos Clavel, Amelie Rezza, Michael Rendl
Developmental & Regenerative Biology, Mount Sinai School of Medicine, New York, NY, United States

Cell-based regenerative therapies are still unavailable to restore hair follicles in hair loss patients and to generate new hair follicles in burn victims or patients with other debilitating skin disorders. Currently, there is a lack of know-how to expand fully functional DP cells in the culture dish for hair inductive cell transplantations. To generate sufficient cell quantities for hair regenerative therapies we sought to reprogram regular fibroblasts into DP cells. Overexpression of previously identified DP signature transcription factors (TFs) in freshly isolated fibroblasts in combination with inhibitors of histone modifiers significantly upregulated several DP signature genes. Furthermore, 3D aggregation clustering of TF overexpressing fibroblast lines isolated from double-transgenic Sox2-GFP/Lef1-RFP reporter mice activated reporter activity and induced the DP molecular identity. Our preliminary data suggest that the right combination of DP TFs can reprogram DP niche fate in regular fibroblasts that can potentially be utilized in future hair restoration efforts.

W-2212

MODELING PARKINSON'S DISEASE USING INDUCED NEURAL PRECURSOR CELLS.

Bronwen J. Connor, Rebecca Pearman, Erin Firmin, Kathryn S. Jones
Pharmacology, University of Auckland,, Auckland, New Zealand

To date, our ability to effectively study and treat Parkinson's disease (PD) has been limited by lack of access to affected human neurons. This restricts the ability to obtain important insights into PD pathology, progression and mechanism, preventing early diagnosis and the development of successful treatment strategies. To overcome this we propose to use direct reprogramming of adult human dermal fibroblasts (HDFs) to generate neural precursor cells and subsequently mature human dopaminergic (DA) neurons to facilitate the study of PD. In support of this proposal, we have demonstrated the ability to transform adult HDFs obtained from PD patients with the LRRK2 mutation directly into neural precursor-like (iNP) cells. Ectopic expression of the neural genes *SOX2* and *PAX6* to either normal- or PD- derived adult HDFs allowed the generation of iNP colonies expressing a wide range of neural stem and pro-neural genes. Most importantly, iNP colonies expressed a range of mesencephalic markers including *ASCL1, NGN2, NURR1, FOXA1, PITX3, NKX2.2* and *LMX1A*. Upon differentiation, iNP cells gave rise to neurons expressing multiple neuronal markers, including the dopaminergic marker tyrosine hydroxylase (TH). To successfully develop a human cell model of PD it is necessary to obtain high yields of dopaminergic neurons. We therefore optimized a protocol for the efficient generation of dopaminergic neuronal cultures from normal and PD iNP cells. This protocol generated an enhanced yield of TH-positive neurons (~50-70%) following differentiation of human iNP cells compared to previous protocols utilizing iPS cells (~10-30%). These results demonstrate that direct reprogramming of

PD patient-derived HDFs to iNP cells provides an efficient mechanism for the generation of dopaminergic neurons for PD modeling and drug development.

W-2213

TRANSCRIPTION FACTORS MYOCD, SRF, MESP1 AND SMARCD3 SIGNIFICANTLY ENHANCE THE CARDIO-INDUCING EFFECT OF GATA4, TBX5, AND MEF2C DURING DIRECT CELLULAR REPROGRAMMING

Nicolas Christoforou[1], Malathi Chellappan[1], Andrew F. Adler[1], Robert D. Kirkton[1], Terry Wu[1], Russell C. Addis[2], Nenad Bursac[1], Kam W. Leong[1]

[1]Biomedical Engineering, Duke University, Durham, NC, United States, [2]University of Pennsylvania, Philadelphia, PA, United States

Transient over-expression of defined combinations of master regulator genes can effectively induce cellular reprogramming: the acquisition of an unpredicted phenotype from a differentiated cell lineage. This can be of particular importance in cardiac regenerative medicine as the heart lacks the capacity to heal itself, but simultaneously contains a large pool of fibroblasts. In this study we used a multi-component experimental approach to determine the cardio-inducing capacity of ten transcription factors to actuate cellular reprogramming of mouse embryonic fibroblasts into cardiomyocyte-like cells: Mesp1, SMARCD3, MYOCD, SRF, NKX2-5, HAND1, HAND2, GATA4, TBX5, MEF2C. Over-expression of transcription factors MYOCD and SRF alone or in conjunction with Mesp1 and SMARCD3 significantly enhanced the basal but necessary cardio-inducing effect of the previously reported GATA4, TBX5, and MEF2C. In particular, combinations of the five or seven transcription factors significantly enhanced the activation of cardiac reporter vectors, and induced an upregulation of cardiac-specific genes. Global gene expression analysis also demonstrated a significantly greater cardio-inducing effect when the transcription factors MYOCD and SRF were utilized. Detection of cross-striated cells was highly dependent on the cell culture conditions and was enhanced by the addition of valproic acid and JAK inhibitor. Importantly, although we detected Ca2+ transient oscillations in the reprogrammed cells, we did not detect significant changes in resting membrane potential or spontaneously contracting cells. This study further elucidates the cardio-inducing effect of the transcriptional networks involved in cardiac cellular reprogramming, with the aim of fine-tuning this approach and ultimately translating into robust cardiac regenerative therapies.

W-2214

SMALL MOLECULE ENHANCED DIRECT CONVERSION OF ADULT DERIVED MOUSE AND HUMAN PATIENT FIBROBLASTS INTO SPINAL MOTOR NEURONS

Andre M. Blumenstein[1], Justin Ichida[2], Esther Son[1], Jeremy Toma[3], Brian Wainger[4], Anthony Arvanites[1], Lance Davidow[1], Kaitlin Sandor[1], Nick Atwater[1], Clifford Woolf[5], Victor Rafuse[3], Kevin Eggan[1], Lee Rubin[1]

[1]Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States, [2]Stem Cell and Regenerative Medicine, USC, Los Angeles, CA, United States, [3]Anatomy and Neurobiology, Dalhousie University, Halifax, NS, United States, [4]Neurobiology, Children's Hospital Boston, Boston, MA, United States, [5]Neurobiology, Harvard Medical School, Cambridge, MA, United States

The mammalian nervous system consists of a large variety of unique neuronal subtypes, some of which are affected in particular neurodegenerative diseases. In the past several years, there has been a great deal of interest in establishing methods of producing large numbers of these neuronal types starting from patient biopsies. Generation of induced pluripotent stem cells (iPSCs) followed by neuronal differentiation is a widely employed technique, but, more recently, direct differentiation methods -generating neurons from fibroblasts without the need for an iPSC intermediate -- have been established. One advantage of direct differentiation is that it can lead to more rapid production of neurons, but this method can be inefficient, especially when starting from adult rodent or human fibroblasts.

We attempted to address this issue by deriving a rapid and effective protocol for producing induced spinal cord motor neurons (iMNs). We performed a chemical screen of 400 selected compounds to identify ones that might capable of improving the number of iMNs that appear as a result of transcription factor mediated reprogramming, starting from tail tip fibroblasts of two-month-old Hb9::GFP mice. After three weeks, compounds were scored based on the appearance of GFP+ iMNs, and secondary assays were then performed on potential hits. We found that small molecule inhibition of Activin signaling improved iMN production over 40-fold by stimulating the survival of both early reprogramming intermediates and of the resulting iMNs. Normal neural progenitors were not generated, though. Surprisingly, the small molecules were as, or more, effective in enhancing the production of human iMNs. In addition, we found that inhibiting this pathway was also effective in helping to stimulate differentiation of neurons of other subtypes, including induced forebrain neurons. The combination of defined transcription factors and small molecule enhancers can now be used in efficient and rapid production of neurons from multiple patient biopsies.
Supported by grants from the NINDS, the Harvard Stem Cell Institute and the New York Stem Cell Foundation.

W-2215

ENHANCED GENERATION OF INDUCED DOPAMINERGIC NEURONS FOR TRANSPLANTATION STUDIES

Ulrich Pfisterer[1], Shane Grealish[1], Maria Pereira[1], Shong Lau[1], James Wood[2], Martin Lundblad[1], Olle Lindvall[2], Malin Parmar[1]

[A]Department of Experimental Medical Sciences, [B]Department of Clinical Sciences, [1]Wallenberg Neuroscience Ctr, Lund, Sweden

Overexpression of three transcription factors Ascl1, Brn2 and Myt1L (ABM) efficiently converts human embryonic as well as post-natal fibroblasts into functional neurons, termed human induced neural (hiN) cells. We have shown that simultaneous expression of the additional transcription factors Lmx1a and Foxa2 results in a dopaminergic cell fate in hiN cells.
Performing a systematic screen and following a novel transduction strategy, a new combination of transcription factors has been identified that leads to a 10-fold increased frequency of DA hiN cell formation from embryonic and postnatal fibroblasts. The function of DA hiN cells in vitro has been determined by DA release and electrophysiology. Current work focuses on the investigation of survival and function of DA hiN cells in rodent models of PD as well as their gene expression profile.
Our data demonstrate an improved strategy to increase the generation of functional DA neurons from human fibroblasts, which survive up to 12 weeks upon grafting into adult rodents. The ability to direct hiN cells towards specific subtypes of neurons at higher frequency as well as to graft reprogrammed cells offers exciting future possibilities to generate patient-specific neurons for disease modeling and brain repair.

W-2216

ENHANCED DIRECT CONVERSION INTO HEPATOCYTES BY ADDITIONAL TRANSCRIPTION FACTOR

Kyung Tae Lim[1], Seung Chan Lee[1], Su Yeon An[2], Yu Jin Jang[2], Jong Hun Kim[1], Kyoung-Jin Oh[3], Tae Hwan Kwak[1], Seung-Hoi Koo[3], Jong-Hoon Kim[2], Dong Wook Han[1]

[1]Dept. of Stem Cell Bilogy, School of Medicine, Konkuk University, Seoul, Korea, Republic of, [2]Lab. of Stem Cell Biology, Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul, Korea, Republic of, [3]Division of Biochemistry and Molecular Biology, Dept. of Molecular Cell Biology and Samsung Biomedical Research Institute, Sungkyunkwan University, Seoul, Korea, Republic of

Recent advances in stem cell biology have shown that terminally differentiated somatic cells can be directly converted to the different types of somatic cells with defined sets of transcription factors without going through a pluripotent state. Recently, it was demonstrated that the combinations of 2 (Hnf4a with one of the Foxa1, Foxa2 and Foxa3) or 3 (Hnf1a/Foxa2/Gata4) hepatic transcription factors could directly convert fibroblasts into functional hep-

atocytes, namely induced hepatocytes (iHeps). However, both the low conversion rates and the unraveled mechanism of direct conversion into iHeps still remain to be elusive. Here, we show that additional transcription factor together with previously described hepatic factors dramatically enhance the direct conversion process into iHeps. Furthermore, in the presence of additional factors, Hnf4a alone could directly induce hepatocyte fate on fibroblasts within 5 days. Directly converted iHeps closely resemble both iHeps generated from the previous combinations and primary hepatocytes in a number of characteristics such as morphology, marker expression, epigenetic status, global gene expression pattern and the functionality. Therefore, our data suggest that additional transcription factor dramatically enhance the direct conversion of fibroblasts into iHeps and also that additional factor could replace some of the hepatic factors.

W-2217

EXPLORING NOVEL PATHWAYS OF NEURAL REPROGRAMMING BY INSTRUCTIVE FACTORS AND PHARMACOLOGICAL INTERVENTION

Marc Thier, Woersdoerfer Philipp, **Frank Edenhofer**
University of Bonn, Bonn, Germany

Transcription factor-driven reprogramming of somatic cells has been shown to yield neurons, cardiomyocytes, neural as well as hepatocyte progenitors, demonstrating that direct conversion of somatic cells developed into a new paradigm for both regenerative medicine and disease modeling. Recently, we demonstrated the direct derivation of neural stem (NS) cells from mouse fibroblasts employing a modified Yamanaka-type reprogramming paradigm. Retroviral transduction of Sox2, Klf4, c-Myc and timely restricted activation of Oct4 was used to initiate dedifferentiation of fibroblast cells and 19 days post infection we observed neurosphere-like colonies that could be readily isolated and clonally expanded both in sphere and adherent cultures. Such induced NS (iNS) cells are able to differentiate into all three neural lineages, neurons, astrocytes as well as oligodendrocytes. Fibroblast-derived iNS cells exhibit clonal growth and maintain their marker expression profile and differentiation capability over prolonged expansion (>50 passages). Transduction of neurogenic transcription factors as well as application of small molecule inhibitors were tested to explore alternative pathways of direct conversion. By this, we generated alternative neural progenitor populations with modified differentiation potentials. Molecular mechanisms underlying reprogramming and therapeutic value of reprogrammed cells will be discussed. We expect converted somatic stem cells such as iNS cells to provide a safe and robust, virtually unlimited source of patient-specific cells for future applications in regenerative medicine and disease modeling.

W-2218

DIRECT CONVERSION OF MOUSE FIBROBLASTS INTO OLIGODENDROCYTE PROGENITOR CELLS BY DEFINED TRANSCRIPTION FACTORS

Hyunah Lee, Myung Rae Park, Seung Hun Lee, Soo Yong Park, Man Sze Wong, Jeong Beom Kim
Max Planck Parter Group - Molecular Biomedicine Laboratory, Hans Schöler Stem Cell Research Center, Ulsan National Institute of Science and Technology, Ulsan, Korea, Republic of

Cell fate can be converted between different somatic lineages without passing pluripotent state. This advanced method provides an alternative to using pluripotent stem cells and rule out the concern of tumorigenicity by undifferentiated pluripotent stem cells during differentiation. Previous studies have shown that defined transcription factors can convert the terminally differentiated somatic cells to other cell types, including induced neural stem cells, hepatocytes, or cardiomyocytes. This strategy provided a new approach toward the regenerative medicine and stem cell therapy for degenerative diseases.
Among many neurodegenerative diseases, oligodendrocyte dysfunction is caused by defect of myelination, including multiple sclerosis, cerebral palsy, and leukodystrophies. Mechanisms these diseases are still unknown and transplantation of myelin-forming cells into injured CNS can be the only possible therapeutics for myelin repair. In this

study, we suggest that generating oligodendrocyte progenitors is potential strategy to develop clinical application for myelin disorders.
Here, we established the efficient derivation of induced oligodendrocyte progenitor cells (iOPCs) from mouse fibroblasts by inducing defined transcription factors. Importantly, these iOPCs are homogenous, self-renewal, and multipotent as somatic stem cells. iOPCs exhibit morphological and molecular characteristics of OPCs such as marker expression. iOPCs can also differentiate into myelin-forming oligodendrocytes expressing mature oligodendrocyte marker as well as in vivo functionality.
We conclude that terminally differentiated cells can be directly converted into functional iOPCs by defined transcription factors. The generation of iOPCs may provide insights into future stem cell therapies for CNS injury and degeneration.

W-2221

REMOTE CONTROL OF NEURONAL FIRING APPLICATION ON MOUSE INDUCED DOPAMINERGIC NEURONAL(IDAN) CELLS

Maria Teresa Dell'Anno[1], Damiana Leo[2], Elena Dvoretskova[2], Ilda Theka[1], Alexander Dytiatev[2], Raul Gainetdinov[2], Bryan Roth[3], Vania Broccoli[1]
[1]Neuroscience, San Raffaele Scientific Institute, Milano, Italy, [2]Neuroscience, Italian Institute of Technology, Genova, Italy, [3]University of North Carolina, Chapell hill, NC, United States

Remote control of neuronal firing may currently be achieved through two well validated approaches: optogenetics and pharmacogenetics. Both the methods allow a detailed analysis of the elecrophysiological properties of discrete neuronal subpopulations genetically modified to express either light-gated ion channels or mutant G-protein coupled receptors, respectively. In the present work we used a pharmacogenetic tool, known as DREADDs, to study the properties of newly reprogrammed cells and compare them with primary cell culture. DREADDs are human muscarinic receptors designed to be a) non- responding to their native ligand and b) activated exclusively by a biologically inert synthetic drug called CNO (Clozapine- N-Oxide). Here two DREADDs known to modulate the neuronal excitability have been investigated: hM3Dq and hM4D. It has already been demonstrated in several neuronal populations that the activation of hM3Dq by CNO promotes the enhancement of neuronal firing, whereas the activation of hM4D induces silencing. We sought to apply DREADDs technology on two kinds of cells: mesencephalic dopaminergic primary neurons and dopaminergic neuronal cells obtained through direct reprogramming (iDAN cells).Whole-cell patch-clamp recordings show that application of CNO on DREADDs expressing cells is able to elicit a significant modulation of action potentials frequency with a similar pattern either in primary cell culture or in iDAN cells.

W-2222

TRANSCRIPTIONAL AND FUNCTIONAL REPROGRAMMING OCCUR WITHOUT CORRESPONDING CHANGES IN DNA METHYLATION DURING THE DIRECT CONVERSION OF FIBROBLASTS INTO MOTOR NEURONS

Justin Ichida[1], Kendell Clement[2], Brandi Davis-Dusenberry[2], Andre Blumenstein[2], Esther Son[2], Jeremy Toma[3], Brian Wainger[4], Samuel Alworth[5], Clifford Woolf[4], Victor Rafuse[3], Lee Rubin[2], Alexander Meissner[2], Kevin Eggan[2]
[1]Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, United States, [2]Harvard University, Cambridge, MA, United States, [3]Dalhousie University, Halifax, NS, Canada, [4]Harvard University, Boston, MA, United States, [5]DR Vision, Seattle, WA, United States

Direct reprogramming provides the intriguing ability to change the identity of a cell while preserving its genetic information. Recent studies suggest select transcriptional regulators can reprogram cells not only to pluripotency, but also to specific somatic cell states, an approach that could have significant translational utility. However, while

several studies have evaluated the integrity of iPSCs, it remains unclear if somatic cells generated by direct reprogramming achieve complete transcriptional, functional, and epigenetic conversion.
To this end, we have evaluated these aspects of lineage conversion in induced motor neurons (iMNs) generated from mouse and human fibroblasts using MN-specific transcription factors. Because iMNs are a specific subtype of neuron that have a well-defined counterpart in mice and humans, we can directly compare their molecular and functional properties.
We first compared the transcriptional profiles of flow-purified Hb9::GFP+ mouse iMNs, bona fide spinal cord MNs, mESC-derived MNs, miPSC-derived MNs, as well as fibroblasts and pluripotent stem cells using genome-wide RNA seq. While all MN samples clustered together, iMNs were more similar to spinal cord MNs (Pearson coefficient = .90) than were stem cell-derived MNs (Pearson coefficient = .85-86). While ~4500 genes were differentially expressed between iMNs and fibroblasts, only 500 genes differed between iMNs and spinal cord MNs and ~700 genes differed between stem cell-derived MNs and spinal cord MNs. Thus, the transcriptional profile of iMNs is highly similar to that of spinal cord MNs while that of stem cell-derived MNs is less similar, indicating that transcriptional reprogramming is essentially complete in iMNs.
To investigate the level of epigenetic reprogramming in iMNs, we mapped their genome-wide DNA methylation profile by bisulfite sequencing. In contrast to transcription, methylation differed significantly between iMNs and spinal cord MNs within and outside of promoter regions while methylation in stem cell-derived MNs was similar to that of spinal cord MNs. During reprogramming, the iMN profile shifted away from the fibroblast pattern but by day 25 was equidistant between fibroblasts and spinal cord MNs. Thus, transcriptional reprogramming precedes and does not require full epigenetic reprogramming.
Next, we evaluated the functional properties of iMNs. They possessed full electrophysiological function and formed neuromuscular junctions in which we readily observed iMN-induced muscle contraction. In addition, mouse iMNs engrafted correctly into chick spinal cord upon transplantation. To evaluate their utility in modeling amyotrophic lateral sclerosis (ALS), in which motor neurons degenerate, we produced human iMNs containing familial ALS mutations in the gene SOD1. In culture, the ALS iMNs degenerated >2-fold faster than those from 12 healthy individuals and exhibited marked hyperexcitability, which has been observed in SOD1 mutant mice and patients and is believed to drive neurodegeneration. Moreover, iMNs from an additional 4/4 familial and 6/21 sporadic patients degenerated rapidly in culture similarly to the SOD1 mutant iMNs. Thus, iMNs possess all measureable functional qualities of motor neurons.
These results show that full transcriptional and functional reprogramming can occur before and without full epigenetic reprogramming during direct conversion to adult somatic cell types.

W-2223

NEW TRANSCRIPTION FACTOR CODES FOR DIRECT REPROGRAMMING OF FIBROBLASTS TO SPECIFIC NEURAL SUBTYPES

Rachel K. Tsunemoto[1], Andrew F. Adler[2], Pavel A. Chubukov[3], Kristin K. Baldwin[3]
[1]University of California San Diego, San Diego, CA, United States, [2]Duke University, Durham, NC, United States, [3]The Scripps Research Institute, La Jolla, CA, United States

Remarkably, recent studies have shown that functional neurons can be generated directly from fibroblasts by ectopically expressing specific combinations of transcription factors or microRNAs. These induced neurons (iNs) will be most useful for disease modeling or therapy if it is possible to control their subtype identity and/or developmental stage in vitro. At present it is not clear how many different neural lineages can be generated by direct reprogramming. To address this question, we have used a mouse line that allows us to screen for the generation of neurons that express the Pcdh21 gene, which is highly enriched in a few rare neuronal subtypes, including the excitatory olfactory bulb mitral and tufted projection neurons. We find that Pcdh21-expressing iNs are not produced using previously published combinations of factors, but that these neurons arise with high efficiency using two new factor combinations. These Pcdh21-expressing iNs exhibit spontaneous and evoked patterns of neural activity and express other genes consistent with a mitral and tufted neuronal identity. We will present additional data to charac-

terize the endogenous neuronal subtypes and developmental stage that these Pcdh21-expressing iNs most closely resemble.

W-2224

INVESTIGATING GENETIC PREDISPOSITION TO ADDICTION WITH INDUCED NICOTINIC NEURONS

Joel W. Blanchard, Kevin T. Eade, Kristin Baldwin
Scripps Research Institute, La Jolla, CA, United States

Tobacco addiction is the leading cause of death in the United States, and is attributed to nearly 5 million deaths worldwide each year. Nicotine is the principle-reinforcing agent in tobacco, exerting its addictive effect on the brain through nicotinic acetylcholine receptors. Genome-wide association studies have identified SNPs in nicotinic acetylcholine receptors that strongly correlate with heavy smoking, nicotine dependence, lung cancer, and heart disease. Mouse studies established that a small midbrain structure, known as the medial habenula (mHb), is responsible for sensing and regulating the amount of nicotine intake, suggesting it may play a major role in human addictions. Although the medial habenula and genetics predispose individuals to nicotine addiction, no cessation therapy selectively targets either. A major limitation to developing mHb and genetically targeted therapies is the absence of in vitro models enabling such studies. To address these limitations we have developed methods employing stem cell technologies that convert mouse fibroblasts to neurons that resemble those of the medial habenula, express nicotinic receptors, and respond to nicotine. We are optimizing these findings by determining the minimal set of transcription factors and growth conditions required for efficiently converting mouse fibroblasts to mature induced nicotinic neurons (iNNs). Once optimal methods for generating iNNs are established, we will examine how common genetic elements that strongly correlate with heavy smoking impair nicotine sensing and response in iNNs. These studies will deliver an in vitro model of medial habenula neurons and establish differences in nicotine sensing and response due to genetic elements known to predispose individuals to nicotine addiction. This work will lay the foundation for future studies aimed at identifying aberrant intracellular signaling pathways in mHb neurons that mediate addiction, and also establish in vitro assays of addiction that can be used to screen for new classes of genetically tailored cessation therapies.

W-2225

HYPOMETHYLATION OF DNMT1 GENE REMOVES THE BARRIER OF IPSC REPROGRAMMING

Julia Chung, Camille Sindhu, Zachary Smith, Atsushi Into, Alexander Meissner, Kevin Eggan
Stem cell and regenerative biology, Harvard University, Cambridge, MA, United States

Reprogramming somatic cells into induced pluirpotent stem cells (iPSCs) by defined transcription factors holds great potential for biomedicine. However, reprogramming process retains slow kinetics and low efficiency. Reprogramming of somatic cells into iPSCs resets the epigenome to embryonic-like state which remains hypomethylation in pluripotentcy genes. We hypothesized that hypomethylation of DNA methyltransferase 1 (Dnmt1) gene might increase the reprogramming potential and perturb a blockade of iPSC reprogramming. Here, we have generated an allelic series of *Dnmt1* MEFs, which have differentially modulated *Dnmt1* expressions, and investigated the reprogramming efficiency. We found that a 90% reduction in the *Dnmt1* level enhances the dynamic of reprogramming over 4-folds, although it causes cell death in hypomorphic MEFs. Furthermore, DNA demethylation synergized with histone demethylation increases their conversion rates up to 10-folds. At the epigenetic level, methylation levels of *Oct4* enhancer are reduced in hypomrophic MEFs, indicating that *Dnmt1* demethylation unravels the repressive effect in the *Oct4* regions. Our findings demonstrate that *Dnmt1*demethylation can increase reprogramming potential of somatic cells. Collectively, our data offer new insights into the nature of epigenetic events inherent to cellular reprogramming.

W-2226

INDUCTION OF PLURIPOTENCY IN PRIMORDIAL GERM CELLS (PGC) BY INHIBITION OF TGFB SIGNALLING PATHWAY

Farnoosh Attari[1], Houri Sepehri[1], Abdolhossein Shahverdi[2], Hossein Baharvand[2]
[1]*Department of Animal Biology, School of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Tehran, Iran, Islamic Republic of,* [2]*Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran, Islamic Republic of*

Introduction: Primordial germ cells (PGCs) have the ability to reprogram into a pluripotent state, named embryonic germ cells (EG) in vitro. In Comparison to iPS cell derivation, the EG cell formation is more efficient and has a shorter culture period than somatic cell reprogramming, furthermore no exogenous genetic manipulations is required in the process. Therefore, EG

cell formation provides a good model to analyze mechanisms by which committed cells acquire pluripotency. So in this study we have tried to induce reprogramming of PGCs toward EG cells with suppressing TGF-β signaling and simultaneous inhibition of ERK pathway with the aim of eliminating usage of GSK3 inhibitor that was previously reported for reprogramming of PGCs. Materials and Methods: To obtain a better robust derivation protocol, we have attempted to identify small molecules (SMs) that induce reprogramming of PGCs in culture. PGCs were isolated and cultured with the presence of different SMs. The emerged EG colonies were picked and expanded in the mentioned medium. The obtained cell lines were further analyzed by immunofluorescent staining, directed differentiation ability and teratoma formation. Results: In this study we have shown that PGCs could be reprogrammed, maintain and expand under feeder and serum free chemically defined conditions by dual inhibition of two signaling pathway and demonstrated that there is no need for GSK3 inhibition for achievement of EG cells from PGCs. Discussion: Here we report that PGCs from different embryo ages (8.5-12.5) can be reprogrammed into pluripotent stem cells through manipulating signaling pathways under chemically modified culture condition, regardless of animal genetic background. Future studies will determine whether generation of EG cells by inhibiting mentioned pathways could be applied to other mammals, especially human and Rat.

W-2227

TRANSFORMATION OF PORCINE PRIMORDIAL GERM CELLS INTO EMBRYONIC GERM CELLS UNDER APPROPRIATE CULTURE CONDITION WITH SMALL MOLECULES

Yanjie Guo, Shiqiang Zhang, Huayan Wang
Dept. Biotechnology, Northwest A&F University, Yangling, China

Embryonic germ (EG) cells are derived from primordial germ cells (PGCs) in vitro under the appropriate culture conditions. EG cells retain the pluripotent and proliferative capacity, which is thought to be equivalent to that of mouse embryonic stem cells. The difficulties of isolating and maintaining EG cells in vitro have restricted their availability for experimental use. To optimize the culture condition, porcine PGCs were isolated from porcine fetus at day 24 of gestation and cultured in media with the different treatments. Three elements, including culture media, feeder cells and small molecules, have been detected to influence the growth of porcine PGCs. When cultured in the basal medium that consists of DMEM supplemented with FBS, LIF, bFGF and SCF for 7 days, PGC clones were investigated by the alkaline phosphatase (AP) activity. The EG cells derived from AP positive colonies represented the naïve-like morphology and were able to propagate on mouse embryonic fibroblasts(MEF) feeder for up to 15 passages, retaining the undifferentiated features. However, EG cells cultured on feeders of porcine embryo fibroblasts (PEF) and stromal cells from mesonephron region, respectively, were unstable and differentiated rapidly. In addition, EG cells were positive for OCT4, SOX2 and SSEA-4, but negative for SSEA-1 by immunofluorescence staining. Quantitative RT-PCR showed that expression of OCT4, NANOG and EpCAM was significantly higher in EG cells than that in porcine somatic cells. We found that the basal medium supplemented with inhibitors of MEK (PD0325901), GSK3β (CHIR99021) and TGFβ (SB431542) could greatly enhance the proliferation of porcine PGC in vitro and clone formation, which were 40 folds higher than that in basal condition. In basal medium added with SB431542 only, the number of PGC clones was elevated ~20 folds. However, basal medium with either PD0325901 or CHIR99021 was just slightly influence the rate of clone formation, suggesting that SB431542 played a critical role in forming the AP positive clones. In summary, we established an efficient culture condition to maintain porcine PGCs growth in vitro, and to facilitate the EG cells derivation and further investigation on porcine pluripotent cells.

W-2228

TARGETED MANIPULATION OF CELL CYCLE AND GENOME STABILITY REGULATORS OVERCOMES RESISTANCE TO REPROGRAMMING IN CELLS FROM FANCONI ANEMIA PATIENTS

Timothy M. Chlon[1], Elizabeth E. Hoskins[1], James M. Wells[2], Susanne I. Wells[1]
[A]Experimental Hematology, [B]Developmental Biology, [1]Cincinnati Children's Hospital, Cincinnati, OH, United States

Fanconi Anemia (FA) is a genetic disease that results from inactivating mutations in one of 15 known genes that are part of a DNA repair pathway. Two recent studies have revealed that cells from FA patients are refractory to somatic cell reprogramming under standard conditions, unless the mutated gene is complemented. One of these studies found that culturing FA cells in hypoxia, which is known to increase reprogramming efficiency in normal cells, also increased the efficiency of iPS colony formation in the context of FA. While an increase in DNA damage and senescence markers was noted during reprogramming in FA cells compared to normal cells, the molecular mechanism by which the FA pathway contributes to reprogramming remains to be elucidated. It is hypothesized that somatic cell reprogramming induces DNA damage, perhaps through the generation of reactive oxygen species (ROS), and that the inability of FA cells to repair this damage results in senescence. The p53 tumor suppressor is known to respond to increased DNA damage by stalling cell cycle progression and even inducing cell death. Inhibition of p53 in normal cells has been shown to dramatically increase the efficiency of reprogramming, indicating that p53 signaling inhibits reprogramming. In this study, we hypothesized that inhibition of p53 or other cell cycle and genome stability regulators would allow FA cells to overcome the block to reprogramming.
We used small molecule inhibitors and retrovirally-expressed oncogenes to target various cell cycle and genome stability regulators during FA cell reprogramming. Our initial results indicate that inhibition of p53, using either the human papillomavirus oncogene E6, the p53 dominant-negative protein p53dd, or p53 specific shRNAs induces iPSc colony formation in FA patient cells. Additionally, treatment with rapamycin or the ROCK inhibitor Y-27632 also induced robust colony formation. Reprogrammed colonies were identified by morphology and positive staining for Tra-1-60 and alkaline phosphatase activity. Our results indicate that multiple pathways can be manipulated to overcome the deficiency of reprogramming in FA cells, and highlight the need to study the consequences of FA pathway loss on cell proliferation and self-renewal in pluripotent stem cells.

W-2231

HIGHER ORDER CHROMATIN STRUCTURE SHAPES THE GENOMIC ABERRATION LANDSCAPE OF PLURIPOTENT STEM CELLS

Junjie Lu[1], Hu Li[2], Ming Hu[3], Jun S. Liu[3], James J. Collins[2], Paul H. Lerou[1]
[1]Department of Newborn Medicine and Division of Genetics, Brigham and Women's Hospital / Harvard Medical School, Boston, MA, United States, [2]Howard Hughes Medical Institute, Department of Biomedical Engineering and Center for BioDynamics, Boston University, Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA, United States, [3]Department of Statistics, Harvard University, Cambridge, MA, United States

Genome organization is intrinsically connected to various functions, including DNA replication, damage repair and genomic aberrations. To investigate the functional effects of genome organization and reorganization during nuclear reprogramming, we mapped one major type of genomic aberration - copy number variations (CNVs) detected by SNP arrays - from low and high passage induced pluripotent stem cells (iPSCs) to megabase-sized chromatin domains defined by DNA replication timing and Hi-C chromatin interactions of the same matched cell types. We found that CNVs are not randomly distributed throughout the genome, consistent with recent reports on the relationship between certain genomic variations in cancer cells and DNA replication timing. In wild type (WT) iPSCs, CNV gains enrich in early replicating domains and open chromatin region while losses enrich in late replicating and closed chromatin regions. However in iPSCs derived from Ataxia-Telangiectasia patients (ATM iPSCs), where DNA damage response and repair (DDR) system is deficient, this trend is markedly different. In low passage ATM iPSCs, gains tend to affect late replicating domains while losses enrich in early domains. However CNVs in higher passage ATM iPSCs have a similar trend as those detected in WT cells. Moreover, we found that the appearance of CNVs is related to the changes in replicating timing program between somatic cells and iPSCs. Our results demonstrate that the dynamic mutational landscape of iPSCs is shaped by genome organization and provide important insights into the effect of genome reorganization and the roles of DDR during reprogramming.

W-2232

GENERATION OF GGTA1 BIALLELIC KNOCKOUT PIGS VIA ZINC-FINGER NUCLEASES AND SOMATIC CELL NUCLEAR TRANSFER

Haide Chen[1], Lei Bao[2], Lei Xiao[1]
[1]*College of Animal Science, Zhejiang University, Hangzhou, China,* [2]*Shanghai Institute of Biochemistry and Cell Biology, Shanghai, China*

Genetically modified pigs are valuable models of human disease and donors of xenotransplanted organs. Conventional gene targeting in pig somatic cells is extremely inefficient. Zinc-finger nuclease (ZFN) technology has been shown to be a powerful tool for efficiently inducing mutations in the genome. However, ZFN-mediated targeting in pigs has rarely been achieved. Here, we used ZFNs to knock out the porcine α-1, 3-galactosyl-transferase (*GGTA1*) gene, which generates Gal epitopes that trigger hyperacute immune rejection in pig-to-human transplantation. Primary pig fibroblasts were transfected with ZFNs targeting the coding region of *GGTA1*. Eighteen mono-allelic and four biallelic cell clones were obtained after drug selection with efficiencies of 23.4% and 5.2%, respectively. The biallelic cells were used to produce cloned pigs via somatic cell nuclear transfer (SCNT). Three *GGTA1* null piglets were born, and one knockout primary fibroblast cell line was established from a cloned fetus. Gal epitopes on *GGTA1* null pig cells were completely eliminated from the cell membrane. Functionally, *GGTA1* knockout cells were protected from complement-mediated immune attacks when incubated with human serum. This study demonstrated that ZFN is an efficient tool in creating gene-modified pigs. *GGTA1* null pigs and *GGTA1* null fetal fibroblasts would benefit research and pig-to-human transplantation.

W-2233

OPTIMISED DERIVATION OF INDUCED PLURIPOTENT STEM CELLS FROM HUMAN AND MOUSE GRANULOCYTES

Jaber Firas, Christian M. Nefzger, Sue Mei Lim, Jose M. Polo
Anatomy and Developmental Biology, Monash University, Melbourne, Australia

Like Embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) are characterised by their ability to differentiate into any cell type of the body. However, unlike ESCs, iPSCs can be derived by reprogramming mature cells via the forced expression of OCT4, SOX2, KLf4 and c-MYC. Accordingly, iPSCs circumvent ethical issues attached to ESC research and have a vast therapeutic potential for disease modelling, pharmaceutical screening and autologous cell therapy. The most common source for iPSC derivation is skin fibroblasts. However, from a clinical point of view, skin fibroblast may not be the ideal cell type, since their acquisition requires an invasive approach such as skin biopsies. Peripheral blood (PB), on the other hand, could be an easy source of patient specific iPSCs because of the availability of minimally invasive collection methods. Granulocytes in particular are highly abundant in PB and constitute 40-60% of all white blood cells. Furthermore, fluorescence activated cell sorting allows the isolation of these cells at high purity, thus iPSCs derivation from granulocytes could provide an alternative to fibroblast derived iPSCs. Previous study succeeded in producing iPSC colonies from murine granulocytes and human mononuclear blood cells but with a low efficiency. Hence, the aim of this study is to increase the efficiency of granulocyte reprogramming into iPSCs from murine and human sources. As granulocytes display poor survival under traditional reprogramming conditions, we investigated the influence of haematopoietic growth factors to stabilise this cell type in vitro and allow for more efficient reprogramming. Granulocytes were isolated from murine bone marrow or human PB and cultured in the presence of a variant of growth factors early or continuously during the reprogramming process. Our preliminary results show that specific growth factors had a significant effect in enhancing the generation of iPSC cells from granulocytes, while other factor combinations had a detrimental effect. In summary, our study has identified haematopoietic cytokine combinations and factors that have a profound effect on the reprogramming of granulocytes. Furthermore, we have devised and optimised a reprogramming protocol for granulocytes. This work can serve as a basis for future work in obtaining large numbers of iPSCs from a clinically relevant cell source.

W-2234

VALIDATION OF A UNIVERSAL INFORMATICS TOOL FOR KINETIC COLONY CHARACTERIZATION IN TIME-LAPSE MICROSCOPY

Zakary A. Kenyon, **Samuel V. Alworth**, James S.J. Lee
DRVision Technologies LLC, Bellevue, WA, United States

The ability to reprogram somatic cells to an embryonic stem cell-like state has had landmark impact on basic biological research, drug screening, and drug discovery. In order to transition induced pluripotent stem cell (iPSC) technology to the clinic for personalized cell therapy, better reprogramming or direct differentiation strategies are needed to eliminate the viral/genetic footprint. Informatics tools are needed to help scientists develop these new strategies by quantifying images of cells and colonies undergoing reprogramming, expansion or differentiation to extract objective and salient features to reliably predict and/or report assay outcomes. Such tools must be flexible for the universal detection of discriminating cellular kinetic and morphological features for different magnifications, seeding densities, protocols and patient types (i.e. sick, healthy).
We have developed an informatics tool for comprehensive colony characterization in time-lapse sequences. The tool provides flexible colony detection that works for phase contrast and fluorescence, different magnifications, protocols and patient types. The tool can characterize colony formation kinetics at very early time-points. The tool can be easily optimized for different cellular kinetics and morphologies using step-by-step user interface that includes machine learning technologies and teach-by-example interfaces. The tool can quantify many aspects of the single colony kinetics including morphological and fluorescence measures, shape and texture.
We have amassed a large database of images showing fibroblast cells undergoing reprogramming and forming colonies. The database includes several magnifications, three reprogramming protocols, different seeding densities, and disease patients and controls. In the present study, we use this image database with annotated truth to validate the performance of the tool's colony characterization. Many parameters are assessed such as detection, segmentation (i.e. colony border definition) and tracking accuracy. Here we present the tools methodology and validation data which shows good performance on a broad range of usage scenarios.
Supported in part by grant number 4R44HL106863 from the NHLBI.

W-2235

AUTOMATIC, LABEL-FREE AND REAL-TIME SELECTION OF IPSC COLONIES AT THE TIME OF COLONY EMERGENCE DURING REPROGRAMMING.

Samuel V. Alworth[1], William T. Hendriks[2], Zakary A. Kenyon[1], Samuel Entwisle[2], Thomas Brickler[2], Chieko Nakada[4], Yasujiro Kiyota[4], Laurence Daheron[3], Lee L. Rubin[2], Chad A. Cowan[2], James S. J. Lee[1]
[1]DRVision Technologies LLC, Bellevue, WA, United States, [A]Department of Stem Cell and Regenerative Biology, [B]Harvard Stem Cell Institute, [2]Harvard University, Cambridge, MA, United States, [3]Nikon Corporation, Tokyo, Japan

The ability to reprogram somatic cells to an embryonic stem cell-like state has had landmark impact on basic biological research, drug screening, and drug discovery. However, picking true induced pluripotent stem cell (iPSC) colonies can be unreliable, costly and time consuming. In particular, currently there are no methods to consistently pick fully reprogrammed iPSC colonies at early time points. Early automated selection of iPSC colonies would greatly reduce the cost and labor of iPSC production, facilitating high-throughput application of iPSC technology. In addition, for researchers less familiar with iPSC technology, computer assisted iPSC colony selection would ease implementation of reprogramming in their laboratories.
Previously, using time-lapse, phase contrast microscopy and kinetic image pattern recognition methods applied to retroviral reprogramming of patient fibroblasts, we uncovered a promising, characteristic pattern of colony formation that can be recognized and used to predict the colonies that would become fully reprogrammed. The approach requires three days of imaging (one image every six hours) and quantitative characterization of colony emergence from the time the colonies are first detected. In practical usage, only a few fully reprogrammed iPS colonies are needed to establish new iPSC lines. Thus, we designed our approach to achieve very high specificity while allowing

lower sensitivity. However, in our previous study we were unable to analyze all of the roughly four thousand colonies which were imaged in the 100 mm dish, and could not be sure of the actual false positive rate of our method. In the current study, we improved the automated image analysis and colony prediction methods so that all colonies' formation kinetics could be analyzed and the true performance assessed. To this end, we employed time-lapse microscopy to image retrovirus- and Sendai virus-mediated reprogramming of healthy and disease-specific fibroblasts, using Klf4, Oct3/4, Sox2 and c-Myc. Here we present our new method and results that show iPS selection within 3 to 5 days of colony emergence with high specificity when applied to a large number of colonies. We believe that this imaging method can ultimately be applied to fully automate large-scale production and selection of IPSCs.
Supported in part by grant number 4R44HL106863 from the NHLBI and the Harvard Stem Cell Institute.

W-2236

CONVERSION OF A RING CHROMOSOME TO UNIPARENTAL DISOMY DURING HUMAN INDUCED PLURIPOTENT STEM CELL GENERATION-IMPLICATIONS FOR DISEASE MODELING AND REPAIR

Marina Bershteyn[1], Yohei Hayashi[2], Edward Hsiao[1], Guillaume Desachy[1], Kathryn Tsang[1], Lauren Weiss[1], Shinya Yamanaka[2], Anthony Wynshaw-Boris[1]

[1]Univ of California, San Francisco, San Francisco, CA, United States, [2]Gladstone Institute of Cardiovascular Disease, San Francisco, CA, United States

Ring chromosomes are found in individuals with mental retardation, seizures and other developmental disorders. Generation of induced pluripotent stem cells (iPSCs) from human fibroblasts with ring chromosomes has not been reported, and it is unclear whether such cytogenetic abnormalities can be modeled with iPSCs. In the course of producing human iPSC models of Miller-Dieker Syndrome (MDS), the most severe form of lissencephaly associated with mental retardation and intractable epilepsy, we tested whether cells from patients with ring chromosomes could undergo reprogramming. MDS is always caused by heterozygous deletions of the most distal region of 17p13.3, with the critical missing region spanning from LIS1 to YWHAE. We used a non-integrating, episomal approach to produce iPSCs from three MDS patients where one of the patients had a ring chromosome 17 with a 17p13.3 deletion (referred to as MDS-1r(17)), while the other two patients (MDS-2 and MDS-3) had similar deletions without a ring chromosome. iPSCs derived from all three MDS patients were functional and exhibited wild type characteristics of self-renewal and differentiation capacity in vitro and in vivo. However, upon differentiation into cortical type neuronal progenitors, MDS-2 and MDS-3 cells exhibited severely impaired proliferation while MDS-1r(17) cells behaved like wild type controls. We investigated the origin of this phenotypic difference and discovered that several independent iPS lines derived from MDS-1r(17) cells had unexpected normal karyotypes without the ring chromosome 17 and surprisingly had wild type levels of LIS1 and YWHAE, in contrast to the 50% reduction of levels of these genes measured in original fibroblasts as well as in iPS lines derived from MDS-2 and MDS-3 patients. SNP arrays revealed two copies of the wild type chromosome 17 in successful iPS clones from MDS-1r(17) fibroblasts, suggesting that compensatory uniparental isodisomy had occurred during the process of reprogramming. Collectively, our results indicate that ring chromosome 17 cannot be modeled in iPSCs as it is incompatible with pluripotent stem cell maintenance. We are in the process of investigating whether this phenomenon is generalizable to other ring chromosomes. Importantly, iPSC reprogramming appears to be a very effective way to correct for this cytogenetic abnormality with important implications for regenerative medicine.

Regeneration Mechanisms

W-2241

- **ASTROGLIAL ACTIVATION BY ENRICHED ENVIRONMENT AFTER TRANSPLANTATION OF MESENCHYMAL STEM CELLS ENHANCES ANGIOGENESIS AND FUNCTIONAL RECOVERY IN CHRONIC HYPOXIC-ISCHEMIC BRAIN INJURY**

JUNG HWA SEO[1], Ji Hea Yu[2], Ji Yong Lee[2], Yang Hyun Cho[2], Sung-Rae Cho[2]

[A]Graduate Program of Nano Science and Technology, [B]Department & Research Institute of Rehabilitation Medicine, [1]Yonsei University, Seoul, Korea, Republic of

Objective: This study aimed to investigate the effects of angiogenesis coupled with astroglial activation by enriched environment(EE) after transplantation of mesenchymal stem cells(MSCs) on neurobehavioral function in an animal model of chronic hypoxic-ischemic (HI) brain injury.

Methods: HI brain damage was induced in 7-day-old CD-1® mice by unilateral carotid artery ligation and exposure to hypoxia(8% O_2 for 90 min). At 6 weeks of age, the mice were randomly injected with either MSCs(1×10^5 cells) or phosphate buffered saline(PBS) into the striatum and assigned to either EE or standard cages(SC), comprising MSC-EE, MSC-SC, PBS-EE, PBS-SC. Whereas SC controls were housed for the same duration in a standard cage without social interaction, EE mice were housed in a huge cage(86×76×31 cm) containing novel objects and running wheels for voluntary exercise, allowing for social interaction(12-15 mice/cage) for up to 2 months. Rotarod, forelimb-use asymmetry, grip strength, and openfeild tests were performed to evaluate neurobehavioral function. We confirmed the fate of transplanted cells, activated astrocytes and glial scar using immunohistochemistry(IHC). To identify growth factors that are regulated by MSC transplantation and/or EE, neostriata separated from brain, a array-based multiplex ELISA assay was used to determine which of the following 10 cytokines or growth factors were detectable in the neostriata. We also evaluated platelet endothelial cell adhesion molecule-1(PECAM-1) and α-smooth muscle actin(α-SMA) using IHC. **Results:** EE after transplantation of MSCs synergistically improved rotarod latency at constant speed of 48 rpm and at accelerating speed ($p<0.05$). In ladder walking test, the percentage of slips was significantly decreased in MSC-EE($p<0.05$). The grip power relative to preoperative evaluation in the contralateral hemiplegic limb showed significant improvements in MSC-EE($p<0.05$). The density of the transplanted cells in the striatum, were 2.34 fold significantly higher in MSC-EE mice than in MSC-SC mice at 2 weeks after treatment ($p<0.05$). Although grafted cells did not differentiate into a large number of neuronal lineage cells, Tuj-1^+ neurons of grafted cells labeled with BrdU were 2.51 times increased in the MSC-EE group than that in the MSC-SC group at 2 weeks after treatment ($p<0.05$). Furthermore, striatal neurogenesis in MSC-EE mice was significantly increased compared to that in the PBS-SC controls in the damaged striata ($p<0.05$). The level of FGF-2 was significantly elevated in mice with EE after transplantation of MSCs at post-treatment 2 weeks ($p<0.001$) and 8 weeks ($p<0.05$). The number of PECAM-1^+ endothelial cells was significantly higher in mice in EE than those in SC after transplantation of MSCs. Two weeks after EE and MSC transplantation, increased densities of PECAM-1^+ and α-SMA^+ cells were coupled with increased $GFAP^+$ astroglial density in the striatum. However, the levels of the glial scar marker CS-56 did not differ among the groups, demonstrating that the combination of EE and MSCs do not increase the detrimental glial scar formation which inhibits neuroregneration after damage. **Conclusion:** EE and MSC transplantation synergistically improved neurobehavioral functions. The underlying mechanisms of this synergism included enhanced repair processes such as higher engraftment of the transplanted MSCs, increased endogenous angiogenesis and astrocyte activation coupled with upregulation of FGF-2.

W-2242

PRIMING BONE MARROW DERIVED MESENCHYMAL STEM CELLS FOR IMPROVED PARACRINE ACTIVITY

Martin J. Stoddart
AO Research Institute Davos, Davos Platz, Switzerland

Introduction: The immediate response to injury is inflammation. The chemotactic and pro-inflammatory factors secreted by different cells following injury are crucial for triggering tissue regeneration. Specifically, these factors induce homing mechanisms of progenitor cells residing in bone marrow or various organs and the release of secondary signaling molecules involved in healing process and cell differentiation. The importance of these factors is often omitted in bone tissue engineering strategies supporting bone regeneration. We aimed to evaluate the influence of these signals on human bone marrow stromal cells.

Methods: Human bone marrow was obtained from four patients (40-65 years old) undergoing hip replacement. Bone marrow stromal cells (MSC) were isolated through Ficoll gradient followed by adhesion to the plastic. Prolif-

erating cells were treated for 2 hours with interleukin 1 β (IL1β; 10ng/ml), Granulocyte colony stimulating Factor (GCSF; 100ng/ml), Stem Cell Factor (SCF; 100 ng/ml) or Stromal Cell-Derived Factor 1 (SDF-1; 1ug/ml) supplemented to the culture medium (DMEM, 10% Fetal Bovine Serum and 1% antibiotics). Then, cells were incubated with fresh medium for following 72 hours. After that time, total RNA was extracted from cells with TRI reagent followed by on-column clean up and gene expression of 84 cytokines was assessed using RT2 Profiler PCR Array according to the manufacturer protocol (Qiagen). The mRNA expression levels of cytokine mRNA in treated cells were normalized relative to untreated cells (control). For each treatment, cells from at least three donors were included.
Results: As expected the extent of gene regulation was affected by donor variability. Nevertheless, consistent effects of stimulation on several genes important in bone regeneration processes were observed. IL-1β treatment induced the up-regulation of genes involved in angiogenesis (IL-6, IL-8) and osteoclastogenesis (TNSF11), while down-regulating genes regulating chondrogenesis (GDF5). Conversely to IL-1β, GCSF negatively regulated expression of gene involved in osteoclastogenesis (INFA1). Indeed, GCSF stimulated expression of anti-inflammatory genes supporting growth of hematopoetic progenitors (IL-8) and bone formation (IL-10). SCF induced the up-regulation of genes regulating cell differentiation (GDF9) and osteoclast differentiation (TNFSF14). Similarly, SDF-1 positively regulated expression of bone formation factors (IL10, BMP8B). Down-regulation of IL-1β was reported in cells treated with SCF and SDF-1.
Discussion: The results of a 2 hour stimulation of human MSCs with IL-1β, GCSF, SCF and SDF-1 demonstrate the influence of these factors on the expression of genes involved in the bone regeneration process. These results were obtained 72 hours after stimulation, suggesting that a short stimulation, which is feasible within an operating theater, has a longer lasting effect during the vital engraftment period. Grouping of these genes regarding their functional activity allows identifying the relation between the stimulating factor and its action required for callus formation, remodeling, angiogenesis, osteoblast and osteoclast differentiation.
Significance: Integrating inflammatory modulation in bone tissue engineering would provide more powerful strategy to enhance bone regeneration processes.

W-2243

ACUTE INFLAMMATION THROUGH LEUKOTRIENE SIGNALING PATHWAY TRIGGERS SPECIFIC REGENERATIVE MOLECULAR PROGRAMS OF THE ADULT ZEBRAFISH BRAIN

Caghan Kizil, Nikos Kyritsis, Michael Brand
Center for Regenerative Therapies (CRTD), TU Dresden, Dresden, Germany

The plasticity of the nervous system in zebrafish is unequalled among vertebrates, for instance regarding the regenerative capacity, which manifests poorly in mammals. The disparity between the regenerative capacities of the central nervous system (CNS) of these animals might be stemmed from the prevalence of specific molecular programs that are turned on specifically after the insult. We previously identified that the injury-induced expression of the transcription factor gata3 is one such specific regeneration readout in zebrafish, as gata3 expression is turned on only after an injury in the radial glial stem cells of the adult zebrafish forebrain, but not in mammalian brains. Using morpholino-mediated gene knockdowns, we found that gata3 was required for injury-stimulated proliferation of the stem cells and subsequent regenerative neurogenesis in fish. Yet, the molecular signalling pathways initiating this specific regeneration program with the injury-induced expression of gata3 was unclear. Therefore, we investigated the initial cues that trigger the regeneration response in adult fish brain. By performing transcriptional profiling experiments on the radial glial cells (RGCs) and developing a novel microinjection method in zebrafish brain to manipulate inflammation, we found that acute inflammation is the cue that triggers specific regeneration programs in zebrafish. To test the effects of acute inflammation, we induced sterile inflammation after injecting fluorescent immunogenic molecules without a traumatic lesion. We showed that this injection elicits a proper neuroinflammation similar to that after the lesion based on the expression dynamics of pro-inflammatory cytokines, leukocyte recruitment, and macrophage morphology. We found that sterile acute inflammation is sufficient to enhance the proliferation of RGCs and neurogenesis without causing any cell death. When we immunosuppressed the fish with Dexamethasone after the lesions, RGC proliferation and regenerative neurogenesis ceased, indicating that inflammation is sufficient and necessary for initiating the regenerative programs in the adult fish brain.

We also discovered that the signaling pathways through the lipid Leukotriene C4 (LTC4) and its receptor cysteinyl leukotriene receptor 1 (cystlr1) initiates RGC proliferation and regenerative neurogenesis. When we blocked the activity of cystlr1 upon lesion with the antagonist Pranlukast, RGC proliferation and regenerative neurogenesis reduced significantly. After administration of LTC4 to the unlesioned fish brains, RGC proliferation and neurogenesis increased through induction of gata3 expression. Thus, we identified that unlike the other vertebrates analyzed so far, acute inflammation has an apparent positive role in triggering the activation of the regeneration-specific molecular programs of the adult zebrafish brain involving gata3. We propose that zebrafish can overcome the detrimental effects of inflammation via special molecular programs linking the non-physiological conditions of traumas to re-development of the lost tissues in part by activating gata3. Our results provide an important fulcrum to the hypothesis that special regenerative programs that enable tissue replenishment might exist in regenerating organisms and understanding such programs would endow us to harness those mechanisms in efforts for clinical regenerative therapies for neurodegenerative disorders or acute injuries in human CNS.

W-2244

WNT SIGNALING IS REQUIRED FOR KIDNEY REGENERATION FROM ADULT ZEBRAFISH NEPHRON PROGENITOR CELLS

Caramai N. Kamei, Yan Liu, Iain A. Drummond
Medicine, Massachusetts General Hospital, Charlestown, MA, United States

Background:
In contrast to mammalian kidney regeneration, zebrafish can not only repair existing nephrons but are also capable of de novo generation of new nephrons from adult organ progenitor cells. Activation of both repair mechanisms is likely to be signaled by positive and negative growth regulators that ultimately restore normal kidney function. We report here that Wnt signaling plays a role in adult zebrafish kidney regeneration.
Methods:
In situ hybridization as well as a transgenic reporter line expressing gfp in regenerating lhx1a-positive nephron progenitors was used to visualize regeneration in response to acute gentamicin injury. Wnt signaling was perturbed using the chemical activator BIO and the inhibitor XAV939.
Results:
Both individual lhx1a-positive adult kidney progenitor cells and differentiating cell condensates are specifically marked by expression of the Wnt receptor frizzled9b (fzd9b). Active Wnt signaling was detected in differentiating nephron progenitors by expression of the canonical Wnt target lef1. Acute injury to the adult zebrafish kidney by gentamicin injection significantly expanded the population of fzd9b-positive nephron progenitors. Pharmacological blockade of Wnt signaling reduced progenitor cell expansion after injury while activation of Wnt signaling was sufficient to expand fzd9b/lhx1a-positive progenitors in the absence of injury.
Conclusions:
Our results demonstrate a role for Wnt signaling in adult zebrafish kidney regeneration and identify fzd9b as a new marker of adult kidney progenitor cells. Our results open new avenues to investigate the developmental origins and regenerative potential of nephrogenic stem cells.

W-2245

ANALYSIS BY LINEAGE TRACING OF ENDOTHELIAL REGENERATION IN MICE FOLLOWING LUNG VASCULAR INJURY INDUCED BY ENDOTOXIN

Min Zhang, Yanmin Zhang, Peter Toth, Xiaojia Huang, Jalees Rehman, Asrar B. Malik
Univ of Illinois at Chicago, Chicago, IL, United States

Acute lung injury (ALI) secondary to lung vascular endothelial injury is a common cause of edema formation and respiratory failure in critically ill patients. Studies have shown that in animal models and patients there is a potential for regeneration of the injured and apoptotic vascular endothelium. However, the primary source of endotheli-

al cells for vascular repair and regeneration remains unclear, and could be driven by proliferation of residual endothelial cells or by the differentiation of stem or progenitor cells into endothelial cells capable of restoring vascular integrity. To answer this question, we performed a Cre/loxP-based lineage-tracing study using tamoxifen inducible Cre-transgenic mice driven by the 5' endothelial enhancer of the stem cell leukemia (SCL) locus. The endothelial-SCL-Cre-ERTAM mouse was bred to a double-fluorescent mT/mG reporter mouse which expressed membrane-targeted tandem dimer Tomato (mT) prior to Cre-mediated excision and membrane-targeted green fluorescent protein (mG) after excision. The vascular endothelial cells were thus GFP+ and non-endothelial cells were Tomato-Red+. We found that the GFP+ endothelial cells were the primary source of endothelial regeneration that occurred 72 hr following lung vascular injury induced by sub-lethal endotoxin lipopolysaccharide (LPS). This was evident by a time-dependent decrease in the number of GFP+ endothelial cells relative to Red+ cells occurring at 12 hr followed by normalization of the ratio at 72 hr (the repair phase). Also, the GFP+ endothelial cells in contrast to the Red+ cells showed 2- to 3- fold increase in proliferation and expression of cyclin genes cyclinF and cyclinA2. These results support the hypothesis that restoration of lung vascular endothelial integrity following LPS is the result of proliferation of pre-existing endothelial cells as opposed to the differentiation of non-endothelial stem or progenitor cells.

W-2246

A NEW APPROACH TO LYMPHATIC DIFFERENTIATION FROM HUMAN PODOPLANIN-POSITIVE MYELOID CELLS FOR ENHANCING LYMPHANGIOGENESIS

Ji-Yeon Yun, Jin Hur, Jin-A Kang, Jae-Il Choi, Young-Eun Choi, Ju-Young Kim, Ji-Hoon Hong, Chang-Hwan Yoon, Sang Eun Lee, Hyun-Jai Cho, Young-Bae Park, Hyo-Soo Kim
Seoul National Univ Hospital, Seoul, Korea, Republic of

Lymphatic vessels are a critical part of the body's immune system. They have a very important role in the pathogenesis of several diseases, such as lymphedema and various inflammatory conditions like attenuated wound healing in diabetes. In order to treat human lymphatic vessel diseases, regeneration of new lymph vessels has attracted academic attention for recent years.
Previous study suggests lymphangiogenesis can occur by mouse
monocyte aggregates transdifferentiating into lymphatic endothelial cells. Emerging studies have also suggested that murine podoplanin-positive myeloid cells are crucially involved in lymphangiogenesis. However, in human conditions, we do not know whether podoplanin-positive myeloid cells could be obtained from peripheral blood and how valuable they are therapeutically. With this study, we investigated the lymphatic differentiation of myeloid cells obtained from human peripheral blood by using aggregate culture condition. Upon closer examination of these human peripheral blood monocyte aggregates, we discovered interesting evidence that the human myeloid cells constituting these aggregates gradually increased the expression of lymphatic endothelial cell markers such as podoplanin and VEGFR-3 during aggregate culture. In particular, monocyte cells sorted for podoplanin markers showed more lymphatic endothelial cell markers such as VEGFR-3, Prox-1, SOX-18 than podoplanin negative cells. The podoplanin-positive cells had a leaf-shape like lymphatic-endothelial cells (LECs) during myeloid aggregates culture and they were also able to participate in LECs in vitro Matrigel. We also confirmed the indirect impact on lymphangiogenesis as well as the direct lymphatic differentiation
ability of podoplanin-positive myeloid cells. The supernatant of them was able to enhance the migration, viability and proliferation of LECs. Local injection of podoplanin-positive cells in monocyte aggregates significantly increased lymphatic neovascularization and facilitated wound healing in the full-thickness skin wounds of mice.
We demonstrated a new approach to induce lymphatic differentiation from podoplanin-positive cells in 3D monocyte aggregate culture system, which would be a promising novel strategy of cell therapy to treat human lymphatic vessel disease.

W-2247

ENHANCED LIGAMENT HEALING USING MESENCHYMAL STEM CELLS

Erin Saether, Connie Chamberlain, Jackie Kondratko, Ellen Leiferman, Stacey Brickson, Wan Ju Li, Ray Vanderby

Orthopedics, University of Wisconsin - Madison, Madison, WI, United States

Objective: Enhance early stage healing in rat medial collateral ligaments (MCLs) using mesenchymal stem cells (MSCs). Specifically, learn how MSCs modulate the inflammatory and immune response upon injury to the MCL.
Methods: A total of 24 rats underwent bilateral MCL transection. Day 5 and day 14 healing was examined comparing two cell doses ($1x10^6$ MSCs and $4x10^6$ MSCs). At the time of surgery, fluorescently-labeled rat MSCs (passage 8-10) were injected into the right transected MCL, while the left MCL served as the control for normal healing. MCLs were collected at the different healing time points and processed with immunohistochemistry (n=12). Type 1 Macrophages (M1), type 2 Macrophages (M2), endothelial cells, proliferating cells, total cells, MSCs, procollagen 1, Collagen 3 and T cells were quantified spatially within the healing ligaments. H+E staining was performed to analyze tissue morphology and the size of the healing region at different time points. Twelve rats underwent MSC injections for mechanical testing.
Results: MSCs were detected solely in the healing region and healing region edges at Day 5 and Day 14 in both dose groups using fluorescence microscopy.
At day 5 healing, the higher dose of cells demonstrated significant changes throughout the ligament with M2's and Procollagen 1. There were more M2's (p=.05) in the distal and proximal healing regions of the normal healing ligament compared to the MSC injection group. Procollagen 1, the precursor to Collagen 1, was increased (p=.05) throughout the MCL that received MSCs.
There were significant changes in both the low dose and high dose groups at day 14. Fewer M1's were found in the ends (p=.01) and throughout the MCL (p=.04) in the low dose group. M2's were decreased in the ends (p=.04), but only in the ligaments that received the higher dose of MSCs.
At day 14, the low dose group had fewer proliferating cells (p=.003) in the healing region compared to the control, whereas in the high dose group there were fewer proliferating cells (p=.05) in the ligament ends.
There were fewer endothelial cells in the ends (p=.03) of the low dose MSC group at day 14, along with fewer lumen in the ends (p=.04) and MCL (p=.002) as a whole.
No significant differences were detected in T cells or Collagen 3.
H+E staining showed a decrease in the length of the healing region in the low dose MSC group. The average length of the healing region in the MSC group was 1.4 mm compared to 2.5 mm (p=.003) in the control ligaments.
By calculating percentage of the healing region size, the low dose group at day14 reached a level of significance (p=.04) with the MSC group's average size representing 8.4% of the ligament and the control 10.4%.
The mechanical results correlated with the healing region size data. The low dose MSC injection group demonstrated increased strength with an average failure load of 26.41N compared to 20.88N in the control group (p=.03). These ligaments also exhibited increased stiffness with an average of 12.24 N/mm compared to 10.01 N/mm (p=.01) in the control ligaments.
Conclusion: MSCs demonstrated a positive healing effect when applied at an appropriate dose shown by a smaller wound size and improved mechanical properties at day 14. Interestingly, the smaller dose of 1 million cells proved to be more successful than the larger dose of 4 million cells. MSCs also affected the cellular response in regard to macrophage phenotype, endothelialization, and proliferating cells in healing MCLs.

W-2248

LOW-FREQUENCY PULSED ELECTRICAL FIELD WITH CERTAIN PARAMETERS INDUCES ERYTHROPOIETIN PRODUCTION, ACTIVATES THE POOL OF MESENCHYMAL STEM CELLS AND ENHANCES REGENERATIVE PROCESSES IN LABORATORY ANIMALS

Mikhail Konoplyannikov[1], Anatoly Konoplyannikov[2]
[1]Federal Research Clinical Center, Moscow, Russian Federation, [2]Medical Radiological Research Center, Obninsk, Russian Federation

Mesenchymal stem cells (MSC) are currently considered as a key component of physiological and "emergency" organism regeneration. We have shown that short-duration action of low-frequency pulsed electrical field with certain parameters onto laboratory animals induces enhanced erythropoietin production, accompanied by increase

in the blood level of reticulocytes and by the activation of the pool of bone marrow MSC. This results in the accelerated healing of wounds and burns, diminishment of radiation damage and other manifestations of enhanced regenerative activity of many tissues. Besides, we have shown that once-per-week treatment of linear mice with low-frequency pulsed electrical field, started at the age of 3 months, statistically significantly extends the animals' life expectancy. It is possible that the observed effects are based on the stimulation of the regenerative potential of MSC due to the erythropoietin action on the specific receptors of the cells. This finding opens new directions for the development of medical technologies of regenerative medicine.

W-2251

VASCULAR ENDOTHELIAL GROWTH FACTOR ENHANCES HUMAN FETAL MESENCHYMAL STEM CELL MIGRATION AND ADHESION

Joan X. Yang, Florentina M. Widodo, Mahesh A. Choolani, Koon Gee Neoh, Jerry KY Chan, Yaw Chyn Lim
National Univ of Singapore, Singapore, Singapore

During injury, chemokines and growth factors (GF) act in concert to recruit different cells types to facilitate repair. Factors such as vascular endothelial growth factor (VEGF) which are produced by endothelial cells (EC) in hypoxic conditions, platelet-derived growth factor (PDGF) which are produced mainly by platelets, and CXCL12/stromal cell-derived factor-1 (SDF-1) are found at high concentrations at inflammatory or injury sites.
Mesenchymal stem cells (MSC) have great potential for therapeutic applications in regenerative medicine. However, despite the numerous reports on MSC migration and homing, their molecular mechanisms have yet to be fully elucidated and understood. Current literature have depicted the importance of the CXCR4-SDF-1 axis in MSC homing and migration. We thus hypothesize that VEGF can modulate the response of human fetal MSC (hfMSC) to SDF-1 and their adhesive capacity at the injury site.
Firstly, we examined hfMSC migration in response to different GF known to be present at the injury site. Except for PDGF (1.30 fold increase against control, $p<0.05$), the other GF did not promote significant migration of hfMSC. We also mimicked the physiological conditions by stressing human umbilical vein EC (HUVEC) under nutrient-deprived conditions and used the conditioned medium (CM) to stimulate hfMSC overnight. We found that CXCR4 expression on CM-treated hfMSC was increased by 1.14 fold ($p<0.05$) compared to untreated controls.
Microparticles (MP) have recently be discovered as a biomarker for various diseases. Endothelial MP (EMP) contain proteins and nucleic acid materials derived from EC. We found that MP derived from stressed HUVEC also augmented CXCR4 expression on hfMSC compared to untreated controls.
As VEGF is a major angiogenic GF secreted during wound healing and possibly also present in the stressed HUVEC CM, we investigated the effects of VEGF on hfMSC migration towards SDF-1. Varying durations of VEGF treatment resulted in differing responses to SDF-1. While 15-minutes treatment did not elicit significantly migration, VEGF treatment for 4 hours resulted in a 1.54 fold increase ($p<0.05$) in hfMSC migration towards 5ng/mL SDF-1 compared to spontaneous migration in the absence of SDF-1. In comparison, untreated cells only showed a 1.12 fold increase in migration in response to SDF-1. 24 hours VEGF treatment also resulted in a significant increase in hfMSC migration, albeit with a smaller magnitude. The treatment, however, did not affect the number of CXCR4 expressing cells nor change the expression of CXCR4 on the cells per se. This suggests that VEGF influence hfMSC response to SDF-1 by modulating the affinity of CXCR4.
Next, we investigated the effect of VEGF treatment on hfMSC adhesion to fibronectin (FN) and vascular cell adhesion molecule-1 (VCAM-1). VEGF treatment augmented hfMSC adhesion to FN and VCAM-1 as compared to bovine serum albumin-coated surface.
Adhesion to FN and VCAM-1 was also dependent on the duration of treatment, with shorter treatments leading to more significant increase in adhesion. Additionally, hfMSC consistently adheres better to FN than to VCAM-1.
Our data supports our hypothesis that recruitment of hfMSC can be modulated by factors, such as VEGF and SDF-1, found at the injury site. hfMSC treated with VEGF *in vitro* augments their migratory response to SDF-1 and binds better to VCAM-1 and FN, thus suggesting that VEGF may be used to augment hfMSC engraftment in tissues.

W-2252

TRANSPLANTATION OF MESENCHYMAL STEM CELLS PROMOTES TISSUE REGENERATION IN A GLAUCOMA MODEL THROUGH LASER-INDUCED PARACRINE FACTOR SECRETION AND PROGENITOR CELL RECRUITMENT

Renaud Manuguerra, Patrick Boulos, Ahmed Ammar, François Leblond, Gorazd Krosl, Vincent Pichette, Mark Lesk, Denis-Claude Roy
University of Montreal / Maisonneuve-Rosemont Research Center, Montreal, QC, Canada

Among bone marrow cells, hematopoietic and mesenchymal components can contribute to repair damaged organs. Such cells are usually used in acute diseases but few options are available for the treatment of chronic disorders. In the present study, we have used a laser-induced model of open angle glaucoma (OAG) to evaluate the potential of bone marrow cell populations and the mechanisms involved in tissue repair. In addition, we investigated laser-induced tissue remodeling as a method of targeting effector cells into damaged tissues. We demonstrate that among bone marrow cells, mesenchymal stem cells (MSC) induce trabecular meshwork (TM) regeneration. MSC injection into the ocular anterior chamber lead to far more efficient decrease in intraocular pressure (IOP)($P<0.001$) and healing than hematopoietic cells. This robust effect was attributable to paracrine factors from stressed MSC, as injection of conditioned medium from MSC exposed to low but not to normal oxygen levels resulted in an immediate decrease in IOP. Moreover, MSC and their secreted factors induced reactivation of a progenitor cell pool found in the ciliary body and increased cellular proliferation. Proliferating cells were observed within the chamber angle for at least 1 month. Laser-induced remodeling was able to target MSC to damaged areas with ensuing specific increases in ocular progenitor cells. Thus, our results identify MSC and their secretum as crucial mediators of tissue repair in OAG through reactivation of local neural progenitors. In addition, laser treatment could represent an appealing strategy to promote MSC-mediated progenitor cell recruitment and tissue repair in chronic diseases.

W-2253

MESENCHYMAL STROMAL CELL (MSC) THERAPY RESTORES RADIATION-INDUCED DYSFUNCTION IN THE NEURAL CONTROL OF COLONIC CIRCULAR MUSCLE CONTRACTIBILITY THROUGH ANTIOXIDANT EFFECTS

Christelle DURAND, Noelle MATHIEU, Christelle DEMARQUAY, Raphaelle BESSOUT, Marc BENDERITTER, **Alexandra SEMONT**
Institut of Radiation protection and Nuclear safety, Fontenay aux Roses, France

Objective: Patients who undergo pelvic or abdominal radiotherapy may develop high incidence of undesirable acute and/or chronic gastrointestinal complications resulting from radiation-induced damages around the tumour. The growing number of cases declared each year and the specific complex symptoms have lead some specialists to talk of a new disease termed “pelvic-radiation disease”. The lack of curative treatment and the potential severity of the disorder highlight the importance of novel and effective therapeutic strategies. In this study, we tested the therapeutic benefit of MSC treatment on radiation-induced dysfunction of colonic circular muscle contractibility (CCMC).
Methods: Rats SD were subjected to a 27 Gy colorectal irradiation. Bone marrow derived MSC (5.106) were intravenously administered three weeks (curative effects) after radiation exposure. Therapeutic effects of MSC were analysed 4 weeks after irradiation. We performed colonic muscle histological (HES) analysis and ex vivo quantification of CCMC in response to escalating doses of Carbachol (non-selective agonist of choline acetyl (Ach), doses: 10-8M to 5.10-4M) with or without inhibition of neural transmission by tetrodotoxin (TTX). Immunostaining of nitrergic neurons (nNO) using neuronal nitric oxyde synthase antibody and cholinergic neurons (nAch) using choline acetyltransferase antibody, were also investigated in myenteric nervous plexus.
Results: Four weeks after irradiation, even if no histological alterations of the circular muscle was observed, we reported a significantly CCMC reduction. Radiation-induced CCMC dysfunction was restored after MSC treatment. Therapeutic effect of MSC is dependent of their ability to restore a normal neutrally induced muscle activity by the re-establishement of the balance between myenteric inhibitory (nNO) and excitatory (nAch) control of CCMC. Indeed, MSC treatment decreased the proportion of myenteric nNO and its activity and increased the proportion of myenteric nAch. This therapeutic effect on enteric nervous system is partially dependent of MSC abilities to reduce oxidative stress via the modulation of glutathione (GSH). Moreover, reduction of CCMC after irradiation was

minimised by the inhibition of lymphocyte T (LT) using a treatment with an anti-CD3. We reported in this study that MSC treatment reduced the activation of LT, mechanism that also might be implicated in MSC therapeutic efficacy on radiation-induced CCMC alterations.
Conclusion: This work constitutes new insights that are arguing in favour of the use of MSC for compassionate applications in order to try to reduce irreversible, pelvic radiotherapy-induced gastrointestinal complications.

W-2254

HEALING A BROKEN HEART; STEM CELL THERAPY FOR DILATED CARDIOMYOAPTHY IN DUCHENNE MUSCULAR DYSTROPHY

Suzanne Berry, Ju Lan Chun, Bob O'Brien, Minho Song
University of Illinois, Champaign, IL, United States

Duchenne muscular dystrophy (DMD) is the most common form of muscular dystrophy, resulting from mutations in the dystrophin gene. Nearly 100% of Duchenne muscular dystrophy (DMD) patients develop dilated cardiomyopathy (DCM) as well severe, progressive pathology in skeletal muscle, and 20-40% succumb to cardiomyopathy. Current methods of management for DCM delay or alleviate decline in cardiac function and remodeling, but will not be curative because they do not restore dystrophin protein or replace damaged cardiac muscle cells. Stem cell therapy offers a mechanism to simultaneously replace damaged cardiomyocytes and restore dystrophin. However, few studies have been conducted to determine whether stem cells will perform these functions in dystrophin-deficient heart and none have previously reported functional benefit.
We have discovered that aorta-derived mesoangioblast stem cells (ADM), with a functional copy of the dystrophin gene, prevent ventricular dilation and a decline in cardiac function in murine models for DMD when transplanted in the heart prior to development of pathology. A number of changes were observed that may account for the prevention of cardiomyopathy. ADM transplantation resulted in the presence of low numbers of dystrophin-positive cardiomyocytes, an increase in vasculature and division of resident nestin+ cardiac stem cells, and the appearance of nestin+ cardiomyocytes of host origin, in dystrophin-deficient heart. It is not yet clear which of these changes is necessary for the observed benefit from ADM. Although angiogenesis in cardiac muscle has been reported to improve heart function following ischemic damage in multiple preclinical and clinical studies, it has not been tested for benefit in dystrophic heart. Dystrophin-deficient cardiomyopathy is not ischemic in origin. However, because an increase in vasculature in our study correlated with functional benefit, an increase in blood flow may also improve cardiac function in DMD. Preliminary studies indicate that the increase in vasculature with ADM transplantation is in the form of vasculogenesis rather than angiogenesis. These data may be useful for developing mechanisms to increase blood flow in dystrophic heart and also suggest that ischemia may be present in dystrophin-deficient heart.
The function of nestin+ cardiac stem cells in normal heart is not known. Our data suggest that resident nestin+ stem cells may be activated to generate new cardiomyocytes in the heart. Consistent with this possibility, a lineage tracing study by another group reported that a subset of nestin+ stem cells in normal adult heart differentiate into cardiomyocytes. We have more recently detected large clusters of nestin+ cardiomyocytes in the heart of 3 different murine models for DMD at the end stages of disease, when extensive pathology is present, even in the absence of ADM injection. Similarly, nestin+ striated cells have also been reported by another research group in the heart of human patients at the end stages of heart failure. Together with this report, our observations of nestin+ striated cells in the dystrophic heart suggest that endogenous stem or progenitor cells may be activated prior to damage, or respond to severe damage in the heart, to generate new cardiomyocytes. We are currently evaluating whether changes in the vasculature and/or activation of nestin+ stem cells may be exploited independently of ADM transplantation to prevent or delay cardiomyopathy in dystrophic heart.

W-2255

TRANSPLANTATION OF PLURIPOTENT STEM CELL DERIVED HUMAN NEURAL PRECURSOR CELLS INTO THE MHV MODEL OF MULTIPLE SCLEROSIS LEADS TO STABLE CLINICAL RECOVERY

Ronald L. Coleman[1], Lu Chen[2], Ronika Leang[2], Suzanne E. Peterson[1], Tom E. Lane[2], Jeanne F. Loring[1]

[1]Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA, United States, [2]Department of Molecular Biology and Biochemistry, University Of California, Irvine, CA, United States

Multiple Sclerosis (MS) is an immune-mediated chronic disease of the central nervous system (CNS) that is characterized by demyelination. Using mice infected with a neurotropic JHM variant of mouse hepatitis virus (JHMV) as a model of MS, we injected human neural precursor cells (hNPCs) derived from human pluripotent stem cells (hPSCs) to explore treatment options for the disease. Two weeks after JHMV infection, hNPCs were transplanted into the spinal cords of mice that exhibited clinical symptoms of the disease. The mice were then scored daily on a clinical diagnostic scale. Mice that were treated with the hNPCs showed significant improvement over mice that received a control treatment as well as mice that had transplantation of human dermal fibroblasts. Additional experiments showed continuing improvement for 3 months post transplantation and sustained recovery for 6 months (the longest duration of the experiment). Some of the mice were sacrificed at 21 days post-transplantation, and their spinal cords were analyzed. Spinal cords of mice treated with hNPCs showed remyelination and decreased macrophage and T-cell infiltration. The decrease in inflammatory cells was correlated with the increase in remyelination.

In order to determine the fate of the transplanted hNPCs, WA09 hES cells were transduced with lentivirus containing an integrating vector with the P. pyralis luciferase gene driven by the cytomegalovirus (CMV) promoter. These cells retained expression of the reporter after differentiation to hNPCs. Mice transplanted with the labeled NPCs were injected with luciferin and imaged daily. No luciferase-expressing hNPCs were detected after day 7 post-transplantation.

To further examine the mechanism of hNPC-mediated recovery, we performed whole genome expression profiling studies on the cells. We identified genes that were differentially expressed in hNPCs relative to fibroblasts and undifferentiated cells. Because of the short survival time of the transplanted cells, we focused on differentially expressed genes coding for secreted proteins. We are assessing these candidates for their potential roles in immunomodulation or oligodendrocyte maturation. The expression of these genes is being verified through quantitative real-time PCR (qRTPCR) and western blot analysis. Genes verified in this manner will be further validated in in vitro and in vivo systems.

Our results show that hNPC transplantation can mediate recovery in a mouse model of MS. Although the hNPCs are rapidly cleared, the mice showed a robust and stable recovery that included remyelination, decrease of inflammation and a lessening of pathological symptoms. Long term, we hope to develop novel stem cell-based therapies to help recovery of MS patients.

W-2256

CO-EXISTENCE OF QUIESCENT AND ACTIVE RADIAL NEURAL STEM CELL POPULATIONS IN THE ADULT HIPPOCAMPUS

Michael A. Bonaguidi[1], Ryan Stadel[2], Yoon Namgung[3], Steven Park[3], Teresa Krieger[6], Jung Jin Oh[3], Guo-li Ming[4], Benjamin D. Simons[6], Hongjun Song[5]

[A]ICE / Neurology, [B]ICE / Human Genetics, [C]ICE, [D]ICE / Neurology / Dept. of Neuroscience, [E]ICE / Neurology / Dept. of Neuroscience / Human Genetics, [1]Johns Hopkins University SOM, Baltimore, MD, United States, [2]Cavendish Laboratory, Dept of Physics/Gurdon Institute, University of Cambridge, Cambridge, United Kingdom

New neurons are continuously generated in the hippocampus throughout life and contribute to specific brain functions. One fundamental question is whether new neurons arise from precursors with characteristics of stem cells or progenitors and whether these precursor properties are uniform. Within the subgranular zone of the adult dentate gyrus, our previous clonal lineage tracing using Nestin::CreERT2 (#) showed that radial glia-like precursors (Nestin#-RGLs) act as quiescent neural stem cells possessing long-term self-renewal and multipotential differentiation capacity. Multiple evidence from population analyses suggests additional cell sources of constitutive neurogenesis in the adult hippocampus. Here, we performed in vivo single-cell lineage-tracing of two RGL subpopulations using the Gli1::CreERT2(#) and Mash1::CreERT2(#) systems. Gli1#-RGLs are initially quiescent but rapidly activate upon label-

ing. These RGLs return to quiescence after either symmetric or asymmetric (neurogenic or astrogliogenic) division. Computational modeling indicates that Gli1#-RGL decisions occur at similar frequencies as Nestin#-RGLs. In contrast, Mash1#-RGLs are already active upon labeling, but primarily undergo neurogenic, asymmetric divisions and undergo long-term self-renewal. Computational modeling suggests that Mash1#-RGLs are biased toward neurogenic self-renewal and represent a distinct unipotential active neural stem cell population. Our study reveals novel insight into the heterogeneity of neural stem cells in the adult brain and supports the co-existence of both quiescent and active neural stem cells in the same somatic compartment where multipotential stem cells cycle between quiescence and activation states, and another more active unipotential stem cell primarily generates neurons.

W-2257

TARGETED ACTIVATION OF PRIMITIVE NEURAL STEM CELLS IN THE MOUSE BRAIN

Rachel Leeder, Brian DeVeale, Cindi M. Morshead, Derek van der Kooy
University of Toronto, Toronto, ON, Canada

Primitive neural stem cells (pNSCs) first appear at embryonic day 5.5 and persist into the adult mouse brain. Primitive NSCs comprise a rare population of GFAP-negative cells that give rise to LIF-dependent neurospheres in culture. They can be passaged in vitro to self-renew or give rise to definitive (d)NSCs, which are GFAP-positive and EGF- and FGF-dependent. Primitive NSCs express low levels of Oct4 and contribute to chimeric blastocysts, both markers of pluripotency not exhibited by dNSCs. We confirmed that pNSCs derived from the pup and adult brain self-renew at the same frequency. We previously observed that pNSCs from the adult brain give rise to equal proportions of neurons, oligodendrocytes and astrocytes following differentiation. Here, we report that pup-derived pNSCs give rise to fewer neurons and more oligodendrocytes than their adult counterparts. This difference in differentiation profiles may reflect the contribution of pNSCs to the wave of oligogenesis that occurs soon after birth. Finally, qPCR to compare pup and adult pNSCs suggests similar gene expression in the two populations. Together, this suggests that pup-derived pNSCs are more abundant, but similar to the adult population, and thus are a valuable population to investigate strategies to isolate and manipulate the pNSC population to better understand its role in the neural lineage. A previous mass spectrometry based screen of in vitro ESC-derived primitive neurospheres identified cell surface markers selectively upregulated in pNSCs compared to ESCs and dNSCs. We are testing these cell surface markers to identify factors that selectively affect the ability of pNSCs to form clonal LIF neurospheres, independent from dNSCs. Specifically, Gleevec, a c-kit pathway inhibitor, significantly increased pNSC neurosphere formation while attenuating dNSC neurosphere formation. Similarly, an ErbB2 inhibitor also selectively increased primitive neurospheres while depleting definitive neurospheres. We confirmed the selective effects of Gleevec using a c-kit siRNA, and the effects of the ErbB2 inhibitor with an ErbB2 siRNA. Next, we infused Gleevec and ErbB2 inhibitor into the lateral ventricle of adult CD1 mice to test the effectiveness in vivo. We observed similar increases in pNSCs following a 3-day infusion and primary dissection to perform a neurosphere assay. Importantly these in vitro to in vivo experiments suggest that markers and inhibitors identified in the pup population can be generalized to adult pNSCs. As well, Gleevec and ErbB2 inhibitors represent two methods of manipulating pNSCs. These methods will enable selective targeting and manipulation of pNSCs to better understand their function in the adult mouse brain.

W-2258

TRANSPLANTED HUMAN NEURAL STEM CELLS RAPIDLY AMELIORATE EARLY DISEASE SYMPTOMS IN A MOUSE MODEL OF CEREBRAL ISCHEMIA

Lei Huang[1], **Jean-Pyo Lee**[2]
[A]Center for stem cell research and regeneration, [1]Tulane Univ Sch of Medicine, New Orleans, LA, United States

Reciprocal interactions occur between neural stem cells (NSCs) and injured host cells. Cerebral ischemia, a common cause of neurologic disability in adults and children, results in loss of cerebral parenchyma and its associated cells and connections. Previously, we exploited cytokines (proinflammatory stem cell factors, which reportedly facilit-

ate stem cell migration into brain injury sites) by demonstrating improved stem cell migration in mid-symptomatic Sandhoff-diseased mice (exhibit lethal gangliosidosis).
Here we assessed the impact of human NSCs in a mouse stroke model by asking the following: Would proinflammatory cytokines promote migration of engrafted NSCs to pathologic sites caused by middle cerebral artery occlusion (MCAO)? Would stem cell-derived factors suppressed proinflammatory genes that function in the breakdown of the blood brain barrier (BBB) and blood cell extravasation into the brain caused by MCAO? We induced MCAO in C57BL6 mice, followed by a 60-minute reperfusion to induce ischemia. NSCs were transplanted intracranially after 24 hours, during the period in which proinflammatory cytokines are upregulated. At 24 hours posttransplantation, transplanted cells indeed migrated extensively and homed to pathologic sites. Cytokines, including TNF-α, IL-6, IL-1β, were downregulated (RT-PCR analysis); Iba1+ active inflammatory cells were significantly decreased (immunostaining); claudin 5, a tight junction protein in blood vessels, was upregulated (Western analysis), suggesting decreased BBB permeability; and infarct volume was decreased (cresyl violet staining). Further, mouse physical behaviors remarkably improved (e.g., rotarod and beam walk tests).
Overall, transplanted NSCs acted swiftly to reduce inflammation and stabilize the BBB following stroke in mice. Thus, our data indicate the potential benefits of NSC transplantation as a therapy following stroke.

W-2261

REJUVENATION OF NEUROGENESIS AND ANGIOGENESIS IN THE AGED BRAIN

Lida Katsimpardi[1], Nadia K. Litterman[1], Pamela A. Schein[1], Christine M. Miller[2], Amy J. Wagers[2], Lee L. Rubin[1]
[1]Stem Cell and Regenerative Biology, Harvard University and Harvard Stem Cell Institute, Cambridge, MA, United States, [2]Stem Cell and Regenerative Biology, Harvard University and Harvard Stem Cell Institute, Cambridge, MA, Howard Hughes Medical Institute and Joslin Diabetes Center, Boston, MA, United States

In the adult mammalian brain, neural stem cells generate new neurons in two discrete areas, the hippocampus and the subventricular zone (SVZ), throughout the lifespan of the animal. Ageing reduces neural stem cell numbers, as well as their self-renewing potential, leading to an overall decline in neurogenesis. However, age-related decline in neurogenesis can be positively regulated by manipulations such as stress and physical exercise, providing evidence that extrinsic factors can delay or even reverse this part of the ageing process. Here we present a study of systemic regulation of SVZ neurogenesis. Using the paradigm of heterochronic parabiosis, where an old and a young mouse are surgically connected so that they share the same circulatory system, we show that the age-related decline of neural stem cell activity in the old mouse is counteracted by systemic factors present in young blood. The pool of neural stem/progenitor cells in the SVZ in the aged brain is increased after exposure to a young circulatory system, leading to an enhancement of olfactory bulb neurogenesis. Importantly, the increase in neurogenesis is associated with a marked increase in the volume of capillaries in the aged SVZ, as well as in other regions of the brain. Unraveling the mechanisms of this phenomenon could have beneficial implications in treating neurodegenerative as well as neurovascular diseases.

W-2262

SUBSTANCE-P MODULATES ENDOTHELIAL PERMEABILITY AND AUGMENTS TRANSENDOTHELIAL MIGRATION OF MESENCHYMAL STEM CELL

Suna Kim, Hyun Sook Hong, Eunkyung Chung, Youngsook Son
Genetic engineering, Kyung Hee University, Yong In, Korea, Republic of

Endothelial cell layer in blood vessel plays an important regulatory role in infiltration of circulating molecules and immune cells to underlying tissue. Substance-P (SP), a member of neuropeptides, is known to mediate pain perception and regulate inflammation. Another point we noticed about SP is a new role as a wound messenger to mobilize mesenchymal stem cells (MSC) from bone marrow that promotes healing mechanisms in various injury models. This study was aimed to reveal early event of the MSC homing mechanism under the influence of SP which might be similar to the SP-treated injured condition. We explored SP's effect on endothelial cells as well as on the

trafficking of MSCs through endothelial monolayer *in vitro*. For measuring endothelial permeability, flux of 40 kDa FITC-dextran from lumen side to basal side was determined in the presence or absence of SP or TNF-α in the lumen side. SP transiently increased FITC-dextran flux, peaked at 5 min and decreased to basal level at 10 min after SP treatment. Based on immunofluorescence staining, SP treatment stimulated the formation of discontinuous and jagged adherens junctions in a multiple interconnected finger-like structure transiently, which was featured by changes in distribution of VE-cadherin and actin cytoskeleton. This SP-mediated gap opening was mostly resealed at 30 min after the SP treatment. In contrast, TNF-α stimulated vascular permeability for much longer duration and completed opening of intercellular junction, which seemed to be mediated by p38 MAPK activation and followed by subsequent induction of VCAM-1 and ICAM-1. Then, we explored whether SP treatment affects trafficking of MSCs. SP stimulated the invasion of MSCs from the lumen side to the basal side through transendothelial migration in 3-D culture system *in vitro*. Collectively, SP directly modulates vascular permeability and homing mechanisms of MSCs through transient regulation of intercellular adherens junction. This study provides the insight into early event of SP-stimulated MSCs homing to the injured tissue, which may be a critical step to explain SP-stimulated tissue repair.

W-2263

LIN28 PROMOTES MAMMALIAN TISSUE REPAIR BY REPROGRAMMING METABOLISM

Ng Shyh-Chang[1], Hao Zhu[2], Tarja Yvanka de Soysa[1], Gen Shinoda[1], Marc T. Seligson[1], Kaloyan Tsanov[1], John Asara[3], Lewis C. Cantley[4], George Q. Daley[1]

[1]Harvard Medical School/Boston Children's Hospital, Boston, MA, United States, [2]UT Southwestern, Dallas, TX, United States, [3]Beth Israel Deaconess Medical Center, Boston, MA, United States, [4]Weill Cornell Medical College/New York Presbyterian Hospital, New York, NY, United States

Robustness of tissue repair and regeneration declines with aging, but the mechanisms relating juvenility and tissue repair remain unclear. The pluripotency factor Lin28, a highly conserved heterochronic gene expressed early in development, controls juvenility and the onset of developmental milestones in animals from worms to humans. In the highly regenerative mammalian liver, genetic deletion of either of the Lin28a/b paralogs impaired oval cell-mediated liver regeneration after chemical injury. To determine if enforced re-expression of Lin28 can promote repair, we examined the effect of overexpressing Lin28a on tissue regeneration in adult mice. After shaving, Lin28a rapidly induced hair regrowth and anagen during the second telogen phase, and after tissue wounding, led to accelerated healing in the adult pinnae and neonatal digits. We assessed the role of let-7, a major downstream target of Lin28, through miRNA gain and loss of function experiments in vivo. Although let-7 overexpression inhibited both liver regeneration and pinnal repair, let-7 knockdown alone failed to enhance tissue repair, suggesting let-7 suppression is necessary but insufficient to account for Lin28-mediated tissue repair. Indeed, we found that Lin28a alters cellular metabolism in vitro and in vivo by directly binding and enhancing the translation of mRNAs for enzymes that mediate glycolysis and oxidative phosphorylation. Surprisingly, pharmacologic inhibition of oxidative phosphorylation abrogated Lin28a-mediated effects without compromising normal tissue repair. Our data indicate that Lin28 enhances tissue repair by programming for a juvenile state of cellular bioenergetics, and suggests that drug modulation of tissue bioenergetics might enhance wound healing.

Tissue Engineering

W-2265

FUNCTIONAL TISSUE ENGINEERED CORNEAL ENDOTHELIUM DERIVED FROM HUMAN IPS CELLS

Satoru Yoshida[1], Shin Hatou[1], Kazunari Higa[2], Miyuki Yasuda[1], Hideyuki Miyashita[1], Kazuo Tsubota[1], Shigeto Shimmura[1]

[1]Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan, [2]Department of Ophthalmology, Tokyo Dental College Ichikawa General Hospital, Chiba, Japan

Corneal endothelial dysfunction remains a common indication for keratoplasty, accounting for half of the total number of such procedures. Keratoplasty using donor cornea includes problems such as immunological rejection. To bypass these problems, we examined application of induced pluripotent stem cells (iPSCs) to engineer artificial corneal endothelium. Corneal endothelium as well as corneal stroma originates from cranial neural crest cells (NCCs). On the basis of these findings, we first tried to differentiate human iPSCs into neural crest cells (iPS-NCCs). Subsequently, the iPS-NCCs were cultured in the specific endothelium-inducing medium. After one week of culture, monolayer of IPSCs-derivd tissue engineered corneal endothelium (iTECE), which shows hexagonal mosaic pattern mimicking the human corneal endothelium was obtained. RT-PCR revealed the expression of characteristic markers of corneal endothelium in iTECE. The markers examined were Na,K-ATPase alpha1-subunit (ATPA1A), carbonic anhydrase 2 (CAR2), Na,HCO3 co-transporter (SLC4A4), collagen IV (CLO4A2), and collagen VIII (COL8A2). We next measured the pump function attributable to Na,K-ATPase activity using an Ussing chamber. As a result, significantly higher Na,K-ATPase pump activity of iTECE sheet compared to that of cultured human corneal endothelial cell sheet, 372.5 μA/cm^2 and 55.9 μA/cm^2 respectively, was observed. Finally, we transplanted iTECE sheets into rabbit cornea to evaluate in vivo pump function of iTECE. The iTECE transplanted rabbit cornea maintained transparency and usual corneal thickness (350 μm), whereas control corneas without TECE showed marked edema and increased corneal thickness (1105.8 μm). Thus, we successfully produced iTECE from human iPSCs, which has sufficient pump function to maintain corneal thickness in vitro and in vivo.

W-2266

DECELLULARIZED CAPRINE TENDON FOR BIOENGINEERING APPLICATIONS IN VITRO

Annie -. John, Thasneem E K Hadi, Francis B. Fernandez
Transmission Electron Microscope Laboratory, Sree Chitra Tirunal Institute for Med. Sciences & Technology, Trivandrum, India

Tendons play a key role in force transmission in the musculoskeletal system and hence are subject to acute forces. The ability of tendons to tolerate repeated force application renders it ideal for use as a decellularized scaffold to replace similar tissues in a biological milieu. On the contrary, tendons are susceptible to load induced tendinopathy, traumatic dissection & similar pathologies and thus a common problem in both occupational and athletic settings. But majority of the tissue is avascular & cell content has low proliferation rates making healing slow post-injury resulting in the formation of inferior scar tissue or fibrous adhesions, which increases the risk of re-injury at the repair site.
Available scaffolds for tendon repair include both biological scaffolds, obtained from mammalian tissues (Alamo tissue service, RTI biologics, etc.), and synthetic scaffolds. Tissue-based scaffolds are an option as ideal biologic scaffolds for de novo construction of damaged tendons. In decellularized tendon tissue the native ECM organization is preserved which provides tolerance for loading at critical points. Tissue engineering by adding stem cells to this pre-prepared scaffold will stimulate the generation of load oriented ECM as well as conditioning of the scaffold to the demands at the site of transplantation.
Caprine tendon tissue (goat Achilles tendon - abattoir sources) was decellularized with Triton-X 100 (1% in Tris buffer, pH-7.4) treatment in combination with continuous agitation and rinses with distilled water and 70% (v/v) ethyl alcohol. Thereafter decellularized tendon scaffold (DTS) along with non-treated fresh tendon scaffold (NTS) was fixed in 10% buffered formalin and 3 % phosphate buffered gluteraldehyde respectively for the study of structural changes if any, after decellularization. Light Microscopy, Scanning and transmission electron microscopy showed characteristic collagen patterns that remained undisturbed after decellularization treatment and absence of cells, nuclei or collagen degradation. NTS and DTS were further subjected to collagenase degradation assay which indicated 43% weight reduction in NTS while in DTS weight reduction was 70%. The lack of cellular inhibition & open architecture as a result of the decellularization process renders the DTS more susceptible to collagenase attack. Contact angle studies showed both surfaces to be hydrophilic (NTS at 49.8 and DTS at 57.4).
Prior to application, it is a prerequisite that decelluarised tendon is non-cytotoxic and cytocompatible. Cytotoxicity (MTT assay with HeLa cells) of the decellularized tissue showed cellular activity well above recommended limits at 24 & 48 hrs. In direct contact assays with L929 fibroblasts, cells attached and grew profusely on the scaffold with

time. In short, the method was successful in creating a non-cytotoxic and cytocompatible ECM scaffold appropriate for tendon bioengineering applications, yet to be validated in a rabbit injured tendon model.
Restoration of normal structure and function of injured tendons represents one of the most challenging areas in Orthopaedics where treatments are largely palliative. Decellularised tendon with stem cells is a promising treatment for the regeneration of injured tendon in the armamentarium of the physician or surgeon.

W-2267

MESENCHYMAL STEM CELLS ATTENUATE HEPATIC FUNCTION IN DECELLULARIZED WHOLE ORGAN SCAFFOLDS TO GENERATE TRANSPLANTABLE LIVER GRAFTS

Yoshie Kadota[1], Hiroshi Yagi[1], Alejandro Soto-Gutierrez[2], Taizo Hibi[1], Yuta Abe[1], Katsunori Tanaka[1], Minoru Kitago[1], Masahiro Shinoda[1], Hideaki Obara[1], Osamu Itano[1], Minoru Tanabe[1], Yuko Kitagawa[1]
[1]Surgery, Keio University, Tokyo, Japan, [2]Surgery, University of Pittsburgh, Pittsburgh, PA, United States

Aim: We assessed the influence of co-perfusion/culture of MSCs (mesenchymal stem cells) with hepatocytes in decellularized liver scaffolds to regenerate transplantable liver grafts which would give the sufficient functionality and viability of hepatocytes.
Methods: We first decellularized the isolated whole rat liver by perfusion of enzyme and non-ionic detergent with the best concentrations for the removal of cellular components and the preservation of native extracellular matrix. Secondly, the acellular translucent matrix scaffold, which was composed of the liver-specific extracellular matrix components and vascular networks, was recellularized with rat hepatocytes and MSCs sequentially via portal vein. To achieve maximum hepatocyte function and viability, we modified; 1) total number of cells, 2) proportion of cells, 3) volume of scaffold, and 4) reseeding methods. Several methods were examined to prevent infection of the perfusion-cultured graft. The two different cell types were tracked to see their locations in the scaffold at different time points by CellTracker Kit and immunofluorescent staining. To evaluate the influence of co-perfusion/culture of MSCs with hepatocytes, we compared the two groups of whole liver scaffolds which were recellularized by; 1) only hepatocytes, 2) hepatocytes and MSCs, of their engraftment and repopulation at day 2, 4 and 6. They were evaluated by histological, biochemical and genetic analyses.
Results: We achieved whole-organ decellularaization for rat livers with the optimal concentration of 0.05% trypsin and 0.1% Triton X-100, which showed the sufficient cell removal and preservation of native structures. The efficient engraftment and survival of hepatocytes and MSCs were demonstrated with a total of 50 X 10^6 hepatocytes and 10 X 10^6 MSCs (20% of hepatocytes), which were mixed and infused into median lobe of the whole liver through portal vein, in three steps, at 2 hours intervals. Also, we could minimize the infection risk of perfusion culture system by antimicrobial and antifungal antibiotics and exposure to ultraviolet-C for the matrix scaffold. Histological findings showed that hepatocytes were aligned as the original hepatic cords from portal vein through central vein. The liver scaffold which was recellularized by co-perfusion/culture of MSCs showed clusters of well-integrated hepatocytes in which MSCs were distributed well with good viability and expressed adhesion molecule markers. In addition, hepatocyte and vascular related growth factors as well as hepatic sinusoid specific markers were expressed along with the decellularized vascular walls, especially around the portal areas of the scaffold, where MSCs were repopulated that was demonstrated by cell tracking method. The graft in which MSCs were co-perfused/cultured showed the less apoptosis of hepatocytes and well-maintained ALB/UREA syntheses as well as higher hepatic gene expressions.
Conclusion: The combination of decellularized whole liver scaffolds and MSCs could show the
potential to create the liver grafts with long-term functionality and viability of engrafted hepatocytes as the result of cell-to-cell and cell-to-matrix interactions that might be catalyzed by their original humoral factors. This regenerated liver graft using MSCs will add new insights for liver regeneration and future clinical use of the decellularized liver matrix scaffold as a bioartificial liver graft.

W-2268

MURINE DECELLULARIZED HEART MATRICES MAINTAIN VIABILITY AND PROLIFERATION OF HUMAN IPS CELLS, BUT ALSO INDUCE THEIR DIFFERENTIATION TOWARDS SPECIFIC CELL FATES

Juliana L. Carvalho[1], Pablo H. Carvalho[2], Carolina R. Oliveira[1], Dawidson A. Gomes[1], Alfredo M. Goes[1]
[A]Biochemistry and Immunology, [B]Clinical and Surgery, [1]Federal University of Minas Gerais, Belo Horizonte, Brazil

Decellularized matrices (DM) are in vogue for tissue engineering applications, due to their favorable chemical composition and perfect tridimensional structure. In addition, it has been described that ECM controls several events in cell biology, such as cell adhesion, proliferation, survival and differentiation. In the present study, we aimed to investigate the biocompatibility of DM and their potential differentiation effects over human iPS cells. Murine decellularized heart matrices (mDhM) were produced through SDS/Triton X-100 protocol, and were tested to: genomic DNA content; microscopic structure and cell content; collagen I and laminin preservation; cytotoxicity; iPS cell adhesion and differentiation induced-effects. Our results indicate that mDhM were virtually 100% decellularized, as no genomic DNA was detected by Nanodrop® quantification and PCR. Corroborating those results, histological analysis performed after Hematoxilin/Eosin, as well as Gomori Trichrome showed no cell nuclei in the matrix, which preserved its microtructure. When used as a scaffold for undifferentiated iPS cells, mDhM induced no significant cytotoxicity, as indicated in MTT assay. iPS cell adhesion wasn't affected by the substitution of Matrigel to mDhM, suggesting its applicability as a scaffold for tissue engineering purposes. Finally, we show that mDhM promote differentiation of human iPS cells, even when cultured in medium for maintenance of their undifferentiated status. Such event was characterized both by PCR and immunofluorescence and underscores the loss of expression of pluripotency markers Oct-4, Nanog and Telomerase reverse transcriptase, as well as the gain of expression of differentiation markers mainly from endoderm (Alpha-fetoprotein and SOX-17) and mesoderm lineages (Brachyuri and cytokeratin-8). Real time PCR is currently being performed in order to determine which embryonic germ layer has been enriched by differential gene expression. Pluripotent stem cells hold great promise for tissue engineering applications due to their high proliferative potential and broad differentiation capacity. Currently, though, the risk of teratoma formation following their injection *in vivo* still hinders their application in the clinic, with only two clinical trials approved by FDA so far. The use of scaffolds for pluripotent stem cell-based tissue engineering applications should, therefore, provide safety measurements for preventing teratoma formation. Herein, we propose that differentiation-inducing scaffolds can be suitable for pluriptent stem cell-based tissue engineering, in order to prevent the injection of undifferentiated cells *in vivo*. In addition, DM provides several classes of differentiation cues, which, sensed by the cells, may promote more successful differentiation event resulting in mature and functional cells.

W-2271

REGENERATION OF VASCULARIZED DENTAL PULP-LIKE TISSUE BY CO-CULTURING DPSCS AND ECS IN A PEPTIDE HYDROGEL SCAFFOLD

Waruna L. Dissanayaka[1], Chengfei Zhang[1], Kenneth M. Hargreaves[2], Lijian Jin[1]
[1]University of Hong Kong, Sai Ying Pun, Hong Kong, [2]University of Texas Health Science Centre at San Antonio, San Antonio, TX, United States

Objectives: To investigate vascular network formation and differentiation of mono- or co-cultured human dental pulp stem cells (DPSCs) and human umbilical vein endothelial cells (HUVECs) within a three-dimensional peptide hydrogel scaffold (PHS); and to utilize this prevascularized construct for dental pulp regeneration in an in-vivo model.
Methods: Monocultures and combinations of co-cultures (3:1, 1:1, 1:3) of DPSCs and HUVECs were encapsulated in different concentrations (0.5%, 0.25%, 0.15%) of PHS (BD-Biosciences, Bedford, MA). DPSCs and HUVECs were transfected with green fluorescent protein and red fluorescent protein respectively using pre-made lentiviral particles (GenTarget Inc, San Diego, CA) before encapsulation. Cellular morphologies and 3-dimensional organization of cultures within PHS were monitored using confocal microscopy. Cell viability was assessed using a live/dead viability assay kit at day 7 and 14. Cell cultures within PHS were induced for odonto/osteogenic differentiation (up

to 21-days), examined for alkaline phosphatase (ALP) activity (ALP quantification assay) and mineralization (Von-Kossa staining). Cell-scaffold constructs were injected into the canal space of tooth-root fragments and implanted into the subcutaneous space on the back of 6- 8-week-old female severe combined immunodeficient mice. Three months after the transplantation, the mice were euthanized and the tooth fragments were removed for histological (Haematoxylin and eosin) and immunohistochemical (human mitochondria and CD31) analysis. Experiments were conducted in triplicate using DPSCs from three different donors and statistically analysed (ANOVA).
Results: Live/dead assay revealed 0.15% as the optimum PHS concentration with a significantly high survival of HUVECs compared to 0.5% and 0.25% PHS. In monocultures, DPSCs survived and grew faster than HUVECs. In co-cultures both cells survived well and HUVECs formed an extensive vessel-like network throughout the PHS compared to HUVEC monocultures where it failed to form any vessel-like structures. This finding suggested that co-culture inhibits HUVECs apoptosis and secretes angiogenic factors to promote vessel-like network formation. At higher endothelial cell counts, the density of vessel-like structures was low and DPSC:HUVEC 3:1 was found as the optimum ratio. ALP activity of cells in co-culture was higher than that of DPSCs in monocultures ($p<0.05$). Despite the ratio, all the co-cultures showed higher amount of mineralization compared to monocultures. The longitudinally sectioned root constructs revealed that 1/3-1/2 of the canals was filled with highly vascularised regenerated pulp-like tissue in both DPSC alone and DPSC-EC co-cultured groups. Majority of the cells in regenerated tissue stained positively for antibodies against human mitochondria confirming the human cell origin of them. Positive staining for CD31 was seen in cells lining the blood vessels in the regenerated tissues, suggesting that the transplanted endothelial cells were responsible for the newly formed vasculature.
Conclusion: These findings indicate that PHS promotes angiogenesis and osteo/odontogenic differentiation in-vitro and can successfully be used for engineering vascularised-pulp tissues in-vivo.

W-2272

ODONTOGENIC DIFFERENTIATION OF DENTAL PULP-DERIVED STEM CELLS ON TRICALCIUM PHOSPHATE SCAFFOLDS

Mohamadreza Baghaban Eslaminejad, Sima Bordbar, Hamid Nazarian
Department of Stem Cell and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran, Tehran, Iran, Islamic Republic of

The regeneration of dental-related tissue is a major problem in dentistry. Thus it is beneficial to develop dental constructs that are fabricated with dental pulp stem cells (DPSCs) and an appropriate scaffold. The present study investigates the level of odontogenic differentiation of human DPSCs on tricalcium phosphate (TCP) scaffolds. Pulp stem cells from human third molars were isolated, culture-expanded through several successive subcultures and characterized in terms of surface markers as well as differentiation potential. The passaged-3 cells were then loaded onto the scaffolds and treated with odontogenic supplements (OS) that included vitamin D3 for a period of 21 days. The scaffolds were Kasios TCP which is commercially available in the form of granules with 3 mm dimensions, average pore size of 200-500 µm and a mean porosity of 60%-80%. DPSCs cultivated on TCP without OS, a monolayer culture treated with OS, and normal pulp tissue were the controls. To determine the loading efficiency, a few hours after cell loading we quantified the cells present within the culture medium. The scaffolds were also prepared for light microscopy (LM) and scanning electron microscopy (SEM) to observe the cells within the scaffold. Furthermore, we compared the groups in terms of odontogenic differentiation markers. According to our results, the loading efficiency was 80%. Based on the representative section prepared from the scaffold/cell construct, DPSCs appeared to occupy the scaffold pore spaces. In SEM images, the cells were observed to establish an attachment with scaffold surfaces. Alkaline phosphatase (ALP) activity, amount of culture mineralization, as well as the expression levels of dentin sialophosphoprotein (DSPP) and dentin matrix acidic phosphoprotein (DMP1) genes tended to be significantly high in the three-dimensional (3-D) cultures treated with OS compared to those 3-D cultures without OS and the monolayer culture with OS ($p<0.05$). The differentiation level of 3-D cultures was considerably lower than that of pulp tissue extracted from third molars ($p<0.05$). 3-D culture on TCP without OS showed a level of differentiation indicating an odontogenic property of TCP biomaterial. In conclusion, the 3-D culture system improves

odontogenic differentiation of DPSCs. The differentiation level of the cells in 3-D culture is significantly lower than that of odontoblasts present in pulp tissue. TCP biomaterial possesses an odontogenic-inducing property.

W-2273

IN VIVO TISSUE FORMATION BY MESENCHYMAL STEM CELLS DERIVED FROM HUMAN EXTRACTED IMMATURE TEETH AND MATURE WISDOM TEETH.

Yuichi Tamaki
Department of Developmental and Regenerative Dentistry, The Nippon Dental University, Tokyo, Japan

Introduction: Mesenchymal stem cells are found in several different dental tissues, including dental pulp, periodontal ligament, apical papilla and dental follicle. In our previous study, we found that DSCs have higher proliferative potential than bone marrow-derived stem cells (BMSC). Dental stem cells (DSCs) have multipotent capacity of differentiate into various cells such as osteoblasts, adipocytes and even neuronal cells. However, it is not clear whether the origin (anatomical or tooth developmental stage) of these cells influences the type of tissue produced. We aimed to investigate the tissue forming potential of DSCs through the transplantation experiments in vivo.
Methods: Stem cells isolated from the apical papilla (APSC) and dental follicle (DFSC) of immature teeth and the periodontal ligament (PDLSC) and dental pulp (DPSC) of mature wisdom teeth, were cultured for three passages before use. Cells were assessed for telomerase activity and expression of periodontal tissue related genes (F-spondin, tenascin-N and periostin) compared with BMSC by Quantitative Telomerase Detection Kit (Biomax) and RT-PCR. To analyze the capacity of the dental stem cells to form hard tissues, we combined each cell type with hydroxyapatite (HA) particles within a collagen gel scaffold. This construct was then transplanted subcutaneously into CB.17 ICR-scid immunocompromised mice. For histological examination, the samples were collected 16 weeks after the transplantation and were fixed with 10% neutral buffered formalin. They were then decalcified, embedded in paraffin, and cut into serial sections (5 μm). The specimens were stained with hematoxylin and eosin and Masson's trichrome. To examine the formed tissue, immunohistochemistry was performed using antibodies against human specific vimentin, periostin, dentin sialoprotein (DSP), and bone sialoprotein (BSP). The primary antibody was omitted during immunostaining as a negative control.
Results: Telomerase activity was higher in APSC and DFSC than in other cell types. Expression of periodontal tissue related genes differences between cell types in F-spondin and tenascin-N expression. DFSC expressed F-spondin higher than the others. Histological analysis showed that all of the DSCs formed a hard tissue structure along with the surface of HA. For immunohistochemical analyses, they were entirely positive for vimentin and periostin antibodies, while they were partially positive for BSP and DSP antibodies.**Conclusions**: These results suggest that DSCs, especially APSC and DFSC, have capacity of forming hard tissues around a suitable scaffold. Thus, immature teeth appear to be an excellent source of cells for targeted dental hard tissue regeneration applications.

W-2274

DERIVATION OF DEFINITIVE ENDODERM -LIKE CELLS FROM HUMAN EYE DERIVED INDUCED PLURIPOTENT STEM CELLS CULTURED IN TWO OR THREE DIMENSIONAL CELL CULTURE ENVIRONMENTS

Mohammad Massumi[1], Elham Hoveizi[2], Farzaneh Pourasgari[3]
[1]National Inst of Genetic Engineering and Biotechnology, Tehran, Iran, Islamic Republic of, [2]Biological Sciences, Tarbiat Moallem University, Tehran, Iran, Islamic Republic of, [3]Biotechnology, Razi Vaccine and Serum Research Center, Karaj, Iran, Islamic Republic of

Due to lake of immunological incompatibility and ethical issues, human induced pluripotent stem cells (hiPSCs) have been considered as potent source for disease modeling, cell replacement therapy and pharmacological screening. In generation of insulin-producing cells from iPSCs, derivation of definitive endoderm (DE) cells is the most critical stage. Furthermore, to generate a specialized tissue *in vitro* that can recapitulate bona fide tissue, culturing and differentiation of cells in a three dimensional (3D) culture environment is required. In the present study, first we established novel iPS cells, ECiPS, which directly reprogrammed from human Eye Conjunctiva-derived Mes-

enchymal Stem Cells (EC-MSCs). To provide 3D culture system PLA/gelatin nanofibrous scaffolds were fabricated. Then ECiPS cells were cultured either on the gelatin (2D) or PLA/gelatin electrospun scaffolds (3D) and induced into DE cell programming fate by specific signaling molecules. Expression analyses of DE-specific markers in mRNA and protein levels confirmed high expression of these markers in ECiPS-derived DE-like cells differentiated in either the 2D or 3D cultural environments. However the level of increase in the ECiPS-derived DE-like cells cultured/differentiated in the PLA/gelatin electrospun scaffolds was significantly higher than expression in the ECiPS-derived DE-like cells cultured/differentiated in the 2D culture system. In conclusion, culturing/differentiation of iPSCs in a 3D culture environment could result in efficient derivation of DE-like cells in comparison to cells cultured/differentiated in a 2D culture system. The result of this study may have impact in future tissue engineering of diseases that implicate DE-derived organs.

W-2275

MOUSE EMBRYONIC STEM CELL-DERIVED IN VITRO MODEL OF HEART, LIVER, AND PANCREAS ON MICROFLUIDIC DEVICE

Yoh-ichi Tagawa[1], Miho Tamai[1], Yoichi Fujiyama[2]

[1]Tokyo Institute of Technology, Yokohama-shi, Japan, [2]Shimadzu Corp., Kyoto, Japan

There are multicellular organisms such as mammalians consisting of a variety of tissues and organs. However, its ontogeny starts from a fertilized egg, a single cell, dividing and differentiating, and finally developing to a single organism having tissues and organs. Blastocyst, a 3.5 day embryo in mouse, consists of trophectoderm and inner cell mass (ICM). The body of the organism is develops from the ICM which can differentiate to three germ-layers. Embryonic stem (ES) cells, having pluripotency to be able to differentiate to three germ-layers, are derived from the ICM.

We have established the murine ES cell-derived *in vitro* model of heart, liver, and pancreas, iHLP^{mES}, consisting of not only beating cardiomyocytes, liver tissue of hepatocytes and endothelial networks, but also pancreatic islets of insulin and glucagon producing cells. The iHLP^{mES} was differentiated from the visceral endoderm-like layer having embryoid body prepared from mouse ES cell lines, E14.1 or ST1. Glucose level in the medium of the iHLP^{mES} model decreased at high insulin, while increased at the high glucagon concentration, suggesting that glucose level could be controlled in the model. Furthermore, multidrug resistance-associated protein 2 and organic anion-transporting polypeptide 2 were confirmed to be functionable in the iHLP^{mES} model, but not in the primary culture of hepatocytes, suggesting that the iHLP^{mES} model was succeeded in having the hepatic multi-polarities. Now, we are trying to equip the iHLP^{mES} with medium-flow system using micro-device like the blood, expecting close to the *in vivo* metabolism system, as a minimal mammal system.

W-2276

HYPOXIC CONDITIONED MEDIUM OF MESENCHYMAL STEM CELLS ENHANCED BONE REGENERATION IN RAT CALVARIAL BONE DEFECTS THROUGH REGULATION OF INTEGRIN α-1 TARGETED-MICRORNA-125B

Yu Jin Jung

Institute of Catholic Integrative Medicine, Incheon St. Mary's Hospital, The Catholic University of Korea., Incheon, Korea, Republic of

Hypoxic conditioned medium of mesenchymal stem cells enhanced bone regeneration in rat calvarial bone defects through regulation of integrin [[Unsupported Character - Symbol Font ]]-1 targeted-microRNA-125b

Yu Jin Jung[a], Hyun-joo Ham[a], Sang In Park[a], Yoonjin Bae[a], Moon-Seo Park[a], Yong-An Chung[a], Lee-So Maeng[a], Woochul Chang[a]

[a]Insitute of catholic Integrative Medicine (ICIM), Incheon St. Mary's Hospital, The Catholic University of Korea.

The use of mesenchymal stem cells (MSCs)-conditioned medium (CM) may be a feasible approach to regenerate bone defect in rat through secreting various factors of MSCs including cytokines, chemokines and growth factors. In this study, we investigated that CM collected under hypoxic culture condition can affect bone regeneration through enhanced migration and adhesion of rat MSCs (rMSCs). rMSCs treated of hypoxic-CM (HCM) showed a significantly increased migration ability compared to the rMSCs treated of normoxic CM (NCM). Also the rMSCs treated of HCM showed increased expression of integrin alpha-1 (ITGA1) which plays a critical role in control of cell migration. The upregulation of ITGA1 was induced by changed expression of the ITGA1 targeted-microRNA-125b on rMSCs because microRNAs are key regulator in various biological functions including cell differentiation, survival and migration by regulating gene expression. Rat calvarial defect model was administrated in three different groups using one of the following graft materials: HCM/greenplast (HCM/g), NCM/greenplast (NCM/g), and DMEM/greenplast (DMEM/g). After 4 and 8 weeks, newly regenerated bone was evaluated using computed tomography (CT) and calcein fluorochrome. CT and calcein analysis indicated that the newly regenerated bone in HCM/g group have enhanced compared to the other groups. These results suggest that HCM may be improved bone regeneration through regulation of ITGA1 targeted-microRNA-125b by secretomes of rMSCs under hypoxic condition

W-2277

HUMAN ENDOMETRIAL MESENCHYMAL STEM CELLS MODULATE THE TISSUE RESPONSE AND MECHANICAL BEHAVIOUR OF POLYAMIDE MESH IMPLANTS FOR PELVIC ORGAN PROLAPSE REPAIR

Caroline E. Gargett[1], Daniela Ulrich[1], Sharol L. Edwards[2], Jacinta F. White[3], Christopher Su[3], Ker Sin Tan[1], Anna Rosamilia[4], John AM Ramshaw[3], Jerome A. Werkmeister[3]

[1]The Ritchie Centre, Monash Institute of Medical Research, Melbourne, Australia, [2]Materials Science and Engineering, CSIRO, Geelong, Australia, [3]Materials Science and Engineering, CSIRO, Melbourne, Australia, [4]Obstetrics and Gynaecology, Monash University, Melbourne, Australia

Pelvic organ prolapse (POP) is the herniation of bladder, bowel and/or uterus into the vagina causing urinary incontinence and sexual dysfunction. POP results from childbirth injury. POP occurs in 25% of all women and 11-19% of women will be treated for POP by reconstructive surgery with or without polypropylene mesh. Complication rates approach 30% and the FDA has posted 2 warnings on the use of polypropylene mesh for vaginal repair surgery. We have identified and characterised human mesenchymal stem cells (MSC) from a novel source, the highly regenerative endometrial lining of the uterus. Perivascular endometrial MSC (eMSC) can be prospectively isolated from endometrial biopsies in the CD140+CD146+ subpopulation (1) or by a single marker, W5C5 (2). Our objective was to use a tissue engineering approach to assess the in vivo biological and biomechanical behaviour of a new synthetic polyamide/gelatin composite mesh (3) seeded with human eMSC in a subcutaneous rat model of wound repair.
Human eMSC were isolated from collagenase-dissociated endometrial tissue using W5C5-labelled magnetic beads, expanded in culture to passage 6, labelled with a fluorescent dye (DiO) and seeded onto fibronectin coated meshes (polyamide/gelatin composite, 25x10 mm) (3) at 500,000 cells/scaffold. Cell-seeded meshes were subcutaneously implanted dorsally in immunocompromised nude rats for 7, 30, 60 and 90 days (n=8/group). Controls received meshes without cells. Explanted samples were analysed by flow cytometry to detect DiO-labelled cells, by immunohistochemistry to assess foreign body reaction and tissue integration. Collagen III/I ratios were quantified by chemical assays, collagen organisation by birefringence and uniaxial tensile testing by Instron.
Implanted meshes were well tolerated and there were no mesh erosions. Labelled eMSC were detected on the mesh up to 14 days post-implant. Meshes with and without cells were equally infiltrated with CD45+ leukocytes, which subsided by 90 days in cell-seeded mesh, but not in mesh alone ($P<0.05$). There were similar temporal differences in CD68+ macrophage infiltration and the M1 and M2 macrophage content differed between mesh seeded with or without cells ($P<0.05$). In cell-seeded mesh, neovascularisation was greater at 7 days than controls ($P<0.05$). New collagen was observed in meshes with and without eMSC. At 7 and 90 days, the meshes seeded with cells were less stiff than those without cells.
This tissue engineering approach reduced the number of inflammatory cells around implanted mesh filaments and promoted neovascularisation, suggesting that eMSC exert an anti-inflammatory effect, promoting wound repair

with minimal fibrosis, and new tissue with more compliant properties. EMSC seeding improves polyamide/gelatin composite mesh biocompatibility and may provide an alternative novel approach for the future treatment of POP.

1.Schwab KE, Gargett CE (2007) Co-expression of two perivascular cell markers isolates mesenchymal stem-like cells from human endometrium. Human Reproduction 22: 2903-11
2.Masuda H, Anwar S, Bühring HJ, Rao J, Gargett CE (2012) A novel marker of human endometrial mesenchymal stem-like cells. Cell Transplantation 21: 2201-14
3.Ulrich D, Edwards SL, White JF, Supit T, Ramshaw JAM, Lo C, Rosamilia A, Werkmeister JA. Gargett CE (2012) A preclinical evaluation of alternative synthetic biomaterials for pelvic organ prolapse repair using a rat abdominal hernia model. PLoS ONE 7 (11):e50044

W-2278

UMBILICAL CORD BLOOD-DERIVED MESENCHYMAL STEM CELLS TRANSPLANTATION WITH THIOLATED HYALURONIC ACID/PEG-DA HYDROGEL IMPROVES CARDIAC FUNCTION IN A RAT MYOCARDIAL INFARCTION MODEL

Byung-Jae Kang[1], Seul Ki Lee[2], Yiming Shen[2], Hwan Kim[4], Sunyoung Jung[3], Nathaniel SukYeon Hwang[4], So Yeong Lee[2], Mincheol Choi[3], Pan Dong Ryu[2], Je-Yoel Cho[1]
[A]Department of Veterinary Biochemistry, [B]Laboratory of Veterinary Pharmacology, [C]Department of Veterinary Medical Imaging, [1]College of Veterinary Medicine, Seoul National University, Seoul, Korea, Republic of, [2]Chemical and Biological Engineering Department, School of Engineering, Seoul National University, Seoul, Korea, Republic of

Mesenchymal stem cell (MSC) transplantation is a newly developed
strategy for cardiac tissue regeneration after myocardial infarction (MI). However, this application is limited by low retention and survival rate of transplanted cells. To improve the efficacy of umbilical cord blood (UCB)-derived MSC therapy for MI, we investigated whether thiolated hyaluronic acid/polyethylene glycol diacrylate (tHA/PEG-DA) hydrogel mixed with UCB-MSC could improve cardiac function and inhibit left ventricle (LV) remodeling in a rat model of MI. tHA/PEG-DA hydrogel alone, MSC alone or tHA/PEG-DA hydrogel seeded with MSC were injected into the infracted myocardium immediately after coronary artery ligation in male rats. Injection of PBS served as control. Five weeks after the MSC transplantation, the echocardiogram showed that injection of UCB-MSC with hydrogel increased the LV ejection fraction and fractional shortening. Histological analysis indicated that injection of UCB-MSC with hydrogel induced a decrease in MI size and an increase in scar thickness, and subsequently prevented scar expansion. This study indicates that tHA/PEG-DA hydrogel could enhance the therapeutic effect of MSC transplantation for MI and therefore the injection of UCB-MSCs with this hydrogel may be a promising strategy in the treatment of MI.

W-2281

BIOLOGICAL BEHAVIOR OF MESENCHYMAL STEM CELLS ON POLI-ε-CAPROLACTONE FILAMENTS AND A POTENTIAL STRATEGY FOR TISSUE ENGINEERING OF PERIPHERAL NERVES

Alvaro C. Ruiz, Rosalia M. Otero, Victor T.R. Resende
Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio De Janeiro, Brazil

Background: Peripheral human nerves fail to regenerate across longer tube implants, most likely because implants lack the microarchitecture of native nerves, including bands of Bungner, that guide selectively regrowing axons. Bone marrow derived cells can contribute to the regeneration of the peripheral nervous system in many aspects under cell therapies approach due to the secretion of soluble factor, for instance. Our previous works showed that microstructured biomaterial filaments are capable of inducing bands of Bungner and that bone marrow cells induce regeneration acting on both neuronal and glial cell populations of the peripheral nervous system (PNS). Therefore, to optimize tubular implants by integrating artificial bands of Bungner and a biological component capable of en-

hancing that regenerative capacity, we study the interaction between poly-caprolactone (PCL) filaments and bone marrow mesenchymal stem cells (MSC).
Methods: For *in vitro* experiments, rat's MSC were placed on PCL filaments previously treated with plasma O_2, poli-D-lysine and laminin. Cells were plated in three different concentrations, $2x10^5$, $2x10^6$ and $8x10^6$ cells/mL, in DMEM F-12 plus 10% fetal bovine serum. After 48h of incubation, the adhesion profile, viability and proliferation capacity of those cells, in contact with the filaments, were analyzed. Adhesion profile was analyzed by immune marking with CD-90 antibody, viability by staining with Calcein AM and Ethidium homodimer-1 and proliferation by immune marking with KI-67 antibody. We also plated rat's adult Schwann cells at the concentration of $5x10^5$ cells/mL on PCL filaments covered with MSC, 24h after the MSC plating, and let the co-culture go on for another 24h to analyze the feasibility of the Schwann cells-MSC-PCL filament system. Before plating, Schwann cells were incubated with CellTrace to assure distinction from the MSC. To test the potential for tissue engineering of peripheral nerves, embryonic rat's dorsal root ganglia were plated in contact with PCL filaments, with or without MSC. The samples were incubated for 4 days then immunolabeled for Neurofilament-200 antibody to analyze neurite extension.
Results: We observed that MSC appeared to be confined to lateral areas and ridges of PCL filaments, showing an alignment along the longitudinal microstructure. However, as we increased the plating concentration of cells, although the number of adherent cells also increased, the rate of alignment gradually decreased, as they agglomerated themselves. Cells showed high viability (90%) and their proliferation capability was not completely inhibited by the filaments. Schwann cells were able to adhere to the filaments plated with MSC maintaining also high viability (90%). Neurites were able to grow and extend over the surface of the PCL filaments when they were previously covered with MSC. Data were analyzed using one-way analysis of variance (ANOVA) with Neuman-Keuls post-test for multiple comparisons.
Conclusion: We provide evidence for the interaction between MSC, Schwann cells and PCL filaments, as they can constitute a stable system permissive for neurite extension and possibly events for the regeneration of the PNS. Moreover, we have ongoing experiments to further refine our study of the interaction between MSC, the peripheral nervous system and PCL filaments.

W-2283

XENO-FREE EXPANSION AND DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS IN STIRRED SUSPENSION MICROCARRIER CULTURES

Yongjia Fan, Emmanuel (Manolis) S. Tzanakakis
Chemical and Biological Engineering, University at Buffalo, Buffalo, NY, United States

A major challenge for the translation of human pluripotent stem cells (hPSCs) to clinical applications -including tissue engineering and cell-based therapies- is the development of scalable bioprocesses affording the generation of medically relevant quantities of cellular material free of xenogeneic factors.
We employed a xeno-free stirred-suspension microcarrier bioreactor for the expansion of undifferentiated hPSCs and their directed commitment to particular fates. A vitronectin-derived peptide was conjugated on the surface of microcarriers. Initially different surface peptide densities were screened in static microcarrier cultures. Subsequently, cells were expanded for five successive passages in stirred suspension. The cells maintained a doubling time similar to that in regular dish cultures, with a 25-fold increase in cell concentration per passage, and exhibited higher than 85% viability. Moreover, the expression levels of pluripotency markers such as Nanog, Oct4 and SSEA4 were preserved as assessed by quantitative PCR, immunochemistry and flow cytometry. Cells cultured on the xeno-free microcarriers exhibited a normal karyotype. After multiple passages in the bioreactor, the cells were subjected to tri-lineage differentiation in static cultures. The resulting cells expressed markers of definitive endoderm (SOX17, FOXA2), mesoderm (MEOX, KDR1) and ectoderm (NES, TUBB3). Lastly, hPSCs propagated on peptide-conjugated beads were directed to mesoderm fate in stirred suspension. The fraction of differentiated progeny co-expressing MEOX1 and KDR was higher than that of cells in dish cultures. These results suggest that the peptide-conjugated microcarrier culture system developed in this study is suitable not only for expansion of self-renewing hPSCs but also for their guided commitment to specific phenotypes. We expect that this culture modality will contribute to

the design and development of stirred-suspension microcarrier bioreactors for the scalable propagation and differentiation of xeno-free hPSCs and their progeny intended for clinical therapies.

W-2284

BUILDING 3D MICROMUSCLE IN VITRO FROM PATTERNED EXTRACELLULAR MATRIX STIFFNESS AND ADIPOSE DERIVED STEM CELLS

Yu Suk Choi[1], Ludovic G. Vincent[1], Andrew R. Lee[1], Matthew G. Ondeck[1], Kyle C. Kretchmer[1], Marek K. Dobke[2], adam J. Engler[1]

[A]Bioengineering, [B]Plastic Surgery, [1]Univ of California, San Diego, La Jolla, CA, United States

ISSCR 2013

Building 3-D µMuscle *in vitro* from Patterned Extracellular Matrix Stiffness and Adipose-derived Stem Cells

Yu Suk Choi, Ludovic Vincent, Andrew Lee, Matthew Ondeck, Kyle Kretchmer, Marek Dobke and Adam Engler

Cell patterning has been widely used to control stem cell shape, size, and adhesion but is typically limited to 2-D via selective deposition of extracellular matrix (ECM); however in tissues, cells are embedded in a 3-D matrix and also influenced by spatial differences in stiffness, which can result in patterned cell arrangements. Indeed, 2-D, fibronectin-coated, mechanically-patterned hydrogels provided more robust and prolonged cell patterns in serum-containing media than their ECM pattern-based counterparts. Adipose-derived stem cell (ASC) differentiation into myocytes was enhanced via patterning through improved alignment and cell fusion rate and number on stiffer regions of the matrix. Conversely, PC12 neuronal cells remain on soft regions of the hydrogel for prolonged culture time, suggesting that mechanical patterning can be used to build micro-tissues *in vitro* to examine more complex structures. To build stiffness-patterned micro-muscle cultures in 3-D, ASCs (or control myocytes) and PC12 neurons were encapsulated in stiffer or softer layers of a metacrylated-hyaluronic acid (MeHa) hydrogel impregnated with RGDS adhesive peptides, respectively. ASCs and myocytes filled stiffer layers with elongated, fused cells while neurons remained within softer layers though they exhibited interactions with myocytes. These data suggest the formation of micro-muscle cultures and the development of new 3-D platforms to create tissue engineered, innervated micro-muscles of neural and muscle phenotypes juxtaposed next to each other.

W-2285

STRETCH-ACTIVATED ION CHANNELS IN HUMAN NEURAL STEM CELL MECHANOTRANSDUCTION

Medha M. Pathak[1], Jamison L. Nourse[2], Truc T. Tran[1], Lisa A. Flanagan[2], Francesco Tombola[1]

[A]Dept. of Physiology & Biophysics, [B]Dept. of Neurology, [1]University of California, Irvine, Irvine, CA, United States

Mechanical properties of the environment, such as rigidity, nanotopology, strain and shear forces are emerging as powerful modulators of stem cell behavior. A recent paradigm shift in stem cell biology has resulted from the discovery that variability in extracellular matrix stiffness is sufficient to direct cell fate and lineage specification. For example, in neural stem cells soft substrates promote neuronal specification whereas hard substrates promote glial specification. Although several molecular players that respond to substrate stiffness at focal adhesion zones have been identified, the mechanisms that direct downstream signaling pathways are not well understood. Here we examine the role of stretch-activated ion channels in matrix-elasticity-driven cell differentiation. Using patch clamp electrophysiological measurements we find that human neural stem/progenitor cells (hNSPCs) exhibit robust membrane-stretch-induced currents. High-speed Total Internal Reflection Fluorescence Microscopy reveals that activity of these channels elicits spontaneous Ca^{2+} transients in hNSPCs that are higher in amplitude on hard surfaces than on soft surfaces. Our results suggest that stretch-activated ion channels may be important for transducing substrate stiffness information into downstream pathways of differentiation in neural stem cells.

W-2286

MECHANICAL AND PHENOTYPIC DYNAMICS OF 3D PLURIPOTENT STEM CELL MORPHOGENESIS

Melissa A. Kinney[1], Rabbia Saeed[1], Pablo A. Hidalgo[2], Boris Zakharin[2], Ari Glezer[2], Todd McDevitt[1]
[1]The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, United States, [2]The George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA, United States

Embryonic stem cells (ESCs) exhibit the capacity for the endogenous cell milieu to direct complex migration and self-organization, including the induction of the epithelial-to-mesenchymal transition, analogous to events mediating primitive streak migration during gastrulation. However, opportunities remain to develop a more complete understanding of dynamics of three-dimensional morphogenesis. The objective of this work is to quantitatively characterize the dynamics of the three dimensional ESC microenvironment, in order to control spatial and temporal aspects of pluripotent cell fate and morphogenesis and ultimately enable the derivation of complex, functional microtissues amenable to the replacement or regeneration of damaged tissue.
The multicellular aggregation kinetics of murine ESCs (D3) were precisely controlled via the combination of forced aggregation and subsequent rotary orbital suspension culture, which enables more uniform and reproducible embryoid body (EB) size, and thereby provides a controlled platform amenable to better understanding three-dimensional stem cell morphogenesis. Supplementation with BMP4 (10 ng/mL) increased expression of mesodermal genes (Gata2, Gata4, VE-cadherin) and induced an epithelial-to-mesenchymal transition, which was apparent within regions exhibiting distinct mesenchymal-like cell morphologies and α-smooth muscle actin (α-SMA) expression. The physical changes accompanying morphogenesis were subsequently analyzed to develop a quantitative understanding of the relationship between EB structure and morphogenesis. Mesenchymal EBs (+BMP4) exhibited significantly increased modulus ($p<0.05$) after 7 days of differentiation compared to those differentiated in basal serum-free media (169.2 ± 8.1, +BMP4; 110.5 ± 4.4 Pa, basal), and dynamically modulated viscoelastic characteristics during differentiation. Cytoskeletal perturbation (Y-27632; 10 μM) significantly decreased the modulus (>1.4-fold decrease), indicating that the stiffness of EBs is mediated in large part by cytoskeletal tension. Moreover, EBs differentiated in the presence of cytoskeletal agonists (jasplakinolide) and antagonists (Y-27632; latrunculin B) exhibited altered stiffness, in a culture condition specific manner; EBs differentiated under basal conditions in the presence of jasplakinolide (actin polymerization) and those treated with BMP4 and latrunculin B (actin depolymerization) exhibited increased stiffness compared to those without cytoskeletal perturbations, suggesting divergent roles for cytoskeletal dynamics in mediating ESC morphogenesis. EBs also exhibited significantly increased fluidic resistance ($p<0.05$ days 4-7 compared to days 2 and 3) during the first 7 days of differentiation, indicating that multicellular structural changes resulting from morphogenesis accompany increasingly limited molecular transport within EBs.
The results of these studies collectively indicate that biophysical properties of morphogenic EB microenvironments, including mechanical and transport characteristics, are dynamically altered as a result of phenotypic changes that transpire during ESC differentiation. Ultimately, understanding biochemical and biophysical tissue morphogenesis is expected to elucidate cellular interactions governing embryonic development and cell specification, and will inform strategies to generate functional tissue structures for regenerative therapies.

W-2287

EVALUATION OF MICROCARRIER-BASED SUSPENSION CULTURES FOR HUMAN INDUCED PLURIPOTENT STEM CELLS

Sara M. Badenes[1], **Tiago G. Fernandes**[1], Annette Pusch[2], Simone Haupt[2], Carlos A.V. Rodrigues[1], Marie-Maud Bear[3], Martial Hervy[3], Maria Margarida Diogo[1], Oliver Brüstle[2], Joaquim M.S. Cabral[1]
[1]Department of Bioengineering, Instituto Superior Técnico, Lisbon, Portugal, [2]LIFE&BRAIN GmbH, Institute of Reconstructive Neurobiology, University of Bonn, Bonn, Germany, [3]CORNING Incorporated, CETC, Avon, France

A microcarrier-based suspension culture was explored for scale-up of human induced pluripotent stem cell (hiPSC) expansion in serum-free medium using synthetic peptide-acrylate surface[1] beads that were developed for long-term support of hiPSC self-renewal. Single-cell inoculation of hiPSCs on the microcarriers was optimized using a chemical inhibitor of the Rho-associated kinase pathway. This microcarrier system supported approximately 10-fold

expansion of the attached cells after 5 days in static culture. Scaling up the microcarrier-based system to 15mL-spinner flasks resulted in a 7-fold increase of the attached cells within 7 days. Initial cell densities of 50,000 and 25,000 cells/cm^2 were tested for a bead concentration of 3 g/l (corresponding to 100 cm^2/spinner). The percentage of cells that attached to microcarriers was very similar in both cases (32-35%). However, reducing the initial cell density resulted in a 2-times higher cell expansion. Envisaging the improvement of the scalability of the culture, multi-passage expansion on the microcarriers was attained by single-cell inoculation of harvested cells and also using confluent microcarriers as the inoculum, which was possible due to bead-to-bead cell transfer. After 4 passages of confluent microcarriers at a 1:1 ratio, a cumulative 339-fold expansion was achieved over 15 days of culture. In static as well as in dynamic cultures cells maintained their typical morphology and pluripotency-associated marker expression. We expect this technology to facilitate the standardized and automated scale-up of hiPSCs and their derivatives for further downstream applications including potential cell replacement therapies.
[1] Melkoumian Z, et al. (2010) Nat. Biotechnol. 28:608–610

W-2288

TISSUE ENGINEERING OF SMALL INTESTINE IN VIVO FOLLOWING SEEDING OF ORGANOID UNITS ON DECELLULARIZED INTESTINAL MATRICES

Panagiotis Maghsoudlou[1], Daniel E. Levin[2], Giorgia Totonelli[1], Allison L. Speer[2], Xiaogang Hou[2], Erik R. Barthel[2], Justin ML Dreyfuss[2], Christa N. Grant[2], Stavros Loukogeorgakis[1], Salvador Garcia[2], Simon Eaton[1], Tracy C. Grikscheit[2], Paolo De Coppi[1]
[1]University College London, London, United Kingdom, [2]Childrens' Hospital Los Angeles, Los Angeles, CA, United States

Aim of the study: Management of intestinal failure remains a clinical challenge and tissue engineering (TE), using an appropriate combination of scaffolds and cells could provide a therapeutic solution. We aim to create a neo-intestine that will be able to be connected to the host's blood supply.
Methods: We decellularized rat intestine using a previously described detergent-enzymatic methodology. We sterilised the scaffolds using UV irradiation, seeded with GFP+ organoid units derived from neonatal mouse intestine and transplanted in the omentum and subcutaneous space of irradiated NOD-SCID mice (n=12). Following 1 month we analyzed the seeded scaffolds using histology and immunofluorescence.
Main results: Following 1 cycle, all of the nuclear material in the scaffolds was removed as evident by histology and DNA quantification. In the luminal aspect, electron microscopy demonstrated an intact crypt-villus micro-architecture. After 1 month of in vivo growth, a number of the seeded rat scaffolds had grown into vascularized balls of 1cm diameter with simple cuboidal epithelium in the luminal side that was E-cadherin+ and villin+.
Conclusion: Transplantation of the rat scaffold in the omentum led to the formation of epithelium. We envisage cell seeding wherein the natural scaffold is surgically micro-anastomosed to the host's blood supply to enhance cell growth.

W-2291

DIRECTED DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO CARDIOMYOCYTES WITH IMPROVED EFFICIENCY IN A DEFINED XENO-FREE SYSTEM

Abhirath Parikh, Emmanuel (Manolis) S. Tzanakakis
Chemical and Biological Engineering, Univ of Buffalo, Buffalo, NY, United States

Embryonic and induced pluripotent stem cells (PSCs) are an inexhaustible source of cardiomyocytes for treating myocardial infarction, a leading cause of morbidity and mortality. Recent advances in the development of patient-specific induced PSCs greatly improve the chances of clinical success. However, the lack of reproducible and efficient methods of cardiac differentiation poses challenges for the development of scalable bioprocesses. Some protocols require additional enrichment or purification steps and most of them are hampered by low differentiation

efficiencies thereby increasing the total operating costs. To resolve these issues, we have assessed different signaling pathways for efficient cardiac differentiation, and investigated methods for improving the yield of cardiomyocytes across a variety of human PSC (hPSC) lines.
Our approach for developing a cardiogenic differentiation method for hPSCs comprises three steps: (1) Stem cells were efficiently directed toward mesoderm-oriented primitive streak in a short timeframe characterized by high expression levels of BRACHYURY (T), MIXL1, EOMES and MESP1 assessed by PCR-gel electrophoresis and quantitative PCR. (2) Subsequently, these cells were coaxed along cardiovascular lineages expressing markers such as KDR, cKIT, Nkx2.5, GATA4, MEF2C. Beating cardiomyocytes were formed within a period of 7 days, and cardiac markers such as β-MHC, TBX20, MLC2a, ANF were strongly upregulated. The upregulation was higher than that seen in cells treated with conventional protocols for cardiogenic differentiation. (3) Finally, further maturation was promoted to cardiomyocytes with an increase in the expression of markers such as MLC2v and α-MHC. Corresponding upregulation of cardiac proteins was observed with western blot analysis. Cells also stained positive for cardiac proteins such as NKX2.5, GATA4, MEF2C, α-ACTININ, cardiac troponins and the cardiac gap junction protein CONNEXIN-43. Functional characteristics were determined by electrophysiology measurements of cardiac action potentials, and response of beating clusters to chronotropic drugs. The efficiency of cardiac differentiation was determined to be up to 70% as assessed by flow cytometry of cardiac markers, and a high number of cardiac cells per stem cell initially seeded was obtained.
Current results for optimizing our differentiation protocol, and for further improving the efficiency are presented and strategies to translate the method to various cell lines are discussed. Parallel work is concentrated on reducing production costs, for example, by introducing small molecules as differentiation factors, for developing scalable processes using stirred suspension vessels to generate functional cardiomyocytes.

W-2292

RAPID HEART DISSOCIATION AND SUBSEQUENT ALLOCATION OF PURIFIED HEART CELLS FOR CARDIAC TISSUE ENGINEERING

Manuel Kernbach, Anne Maria Wiencierz, Andreas Bosio, Dominik Eckardt, **Peter Christalla**
Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Engineered cardiac tissue offers the possibility to replace infarcted myocardium and to investigate heart development and cardiomyogenesis in a simplified in vitro model. It is generally accepted that tri-culture of cardiomyocytes (CMs), fibroblasts (Fibs) and endothelial cells (Endos) enhances functionality of engineered cardiac tissues in vitro and improves survival in vivo. However, one of the major challenges is the availability of pure heart cells to generate in vivo-like cardiac tissues in a dish. Utilizing an automated tissue dissociator (gentleMACS) we developed a rapid neonatal heart dissociation protocol enabling an efficient immunomagnetic enrichment of CMs, Fibs and Endos.
First, we established an enzyme mix optimal for the dissociation of neonatal mouse and rat hearts (P0-P3) by screening our enzyme library. Next, we optimized the dissociation by utilizing our gentleMACS technology resulting in a fast (1h), robust and automated heart dissociation protocol. Analysis of the dissociated heart cells showed: (i) high cell vitalities (>90%), (ii) high frequencies of α-actinin-positive CMs (60%; P2) and (iii) vimentin-positive non-CMs with a frequency of 40% (P2). In order to selectively enrich various cell-types from these heterogenous cell populations, we performed a cell-surface marker screen. We identified several candidates for the composition of antibody cocktails enabling selective enrichment of CMs, Fibs or Endos with purities of up to 97%. Cultured CMs showed spontaneous beating activity and the expression of sarcomeric proteins in a cross striated pattern. In summary, we established an automated protocol for the dissociation of neonatal hearts, enabling the subsequent immunomagnetic enrichment of CMs, Fibs and Endos which can readily be utilized to generate in vitro heart muscle models and surrogate tissue for myocardial repair.

Embryonic Stem Cell Pluripotency

W-2295

CULTURE OF HUMAN ES AND IPS CELLS USING TESR™-E8™: A SIMPLIFIED AND LOW PROTEIN MAINTENANCE MEDIUM COMPATIBLE WITH EITHER BD MATRIGEL™ OR VITRONECTIN XF™

Erik B. Hadley, Alvin Ng, Heather Drew, Matthew Wong, Mandy Chan, Jessica Norberg, Melanie D. Kardel, Jennifer Antonchuk, Cindy Miller, Allen C. Eaves, Terry E. Thomas, Sharon A. Louis
STEMCELL Technologies Inc., Vancouver, BC, Canada

The media, protocols and culture systems used for human embryonic stem (ES) and induced pluripotent stem (iPS) cells under feeder-free conditions have undergone steady improvements since their widespread use was adopted during the last decade. A key contributor to this progress has been the defined, reproducible and feeder-free maintenance medium and culture conditions provided by commercially available mTeSR™1 (STEMCELL), which was developed based on publications from James Thomson's laboratory [Ludwig et al., 2006]. Further developments by the Thomson laboratory have demonstrated that it is possible to remove some extraneous additives and most of the protein content from the base mTeSR™1 formulation, resulting in a simplified medium known as E8 [Chen et al., 2011]. A commercially manufactured version of the E8 formulation, TeSR™-E8™ (STEMCELL), is now available as an alternative maintenance medium for human ES and iPS cell culture. The aim of this study was to test the robustness of new TeSR™-E8™ by assessing cell morphology, cell expansion and pluripotent marker expression in two ES (H1, H9) and three iPS (WLS-1C, WLS-4D1, A13700) cell lines cultured in TeSR™-E8™ in the presence of two different culture matrices: BD Matrigel™ and Vitronectin XF™ (Developed and manufactured by Primorigen Biosciences). To initiate TeSR™-E8™ cultures, cells routinely cultured in mTeSR™1 were dissociated into cell aggregates and then plated onto pre-coated plates containing TeSR™-E8™ medium. Cells were passaged every 5 - 7 days for up to 20 passages using an enzyme-free passaging protocol. Briefly, cells were dissociated using Gentle Cell Dissociation Reagent (STEMCELL) for either 6 - 8 minutes (BD Matrigel™) or 10 - 12 minutes (Vitronectin XF™). The dissociation reagent was removed and replaced with TeSR™-E8™ medium prior to detachment of cell aggregates by scraping. A key step in the passaging protocol was the generation of appropriately sized cell aggregates after harvest by controlling the mechanical dissociation steps prior to replating of the cells. We observed that colonies grown in TeSR™-E8™ had a more condensed and round morphology when grown on Vitronectin XF™ compared to colonies grown on BD Matrigel™, which were more diffuse and irregularly shaped. High cell expansion was achieved across the ES and iPS cell lines tested over the indicated number of passages (p) when using either BD Matrigel™ (H1: 21 ± 3.6 fold; 10p, H9: 36 ± 5; 10p, WLS-1C: 35 ± 5; 10p, WLS-4D1: 19 ± 3; 10p, A13700: 30 ± 4; 10p) or Vitronectin XF™ (H1: 28 ± 2 fold; 6p, H9: 33 ± 4; 8p, WLS-1C: 34 ± 4; 4p, WLS-4D1: 37 ± 7; 7p, A13700: 56 ± 20; 4p). Finally, high expression of pluripotent markers (SSEA3, TRA-1-81 and Oct4) was maintained in cells cultured in TeSR™-E8™ on both matrices. In conclusion, our findings demonstrated that TeSR™-E8™ is a robust maintenance medium for human ES and iPS cells, and is capable of maintaining high quality cell cultures in combination with either BD Matrigel™ or defined Vitronectin XF™.

W-2296

MAINTENANCE OF PLURIPOTENT STEM CELLS CONTROLLED BY KLF5

Takuya Azami, Ken Matsumoto, Hyojung Jeon, Satoru Takahashi, Masatsugu Ema
Department of Anatomy and Embryology, University of Tsukuba, Tsukuba, Japan

Pluripotency is maintained by the core transcription factors, such as Oct4, Sox2, and Nanog. These transcription factors also have important roles in embryonic development and can reprogram somatic cells into iPS cells. Among the Yamanaka factors, Klf4 is dispensable for ES cells self-renewal and early embryo development. Klf4 together with Klf2 and Klf5 play redundant functions in ES cells. However, our previous study clearly indicated that Klf5 is indispensible for blastocyst development (Ema et al., 2008). Therefore, it is important to elucidate the molecular mechanism underlying these functions.
To understand the mechanism, we first addressed the precise phenotype of Klf5 KO embryos. By investigating the BrdU incorporation activity and lineage-marker expressions in Klf5 KO embryos at each early development stage, we show that cell cycle progression is impaired before the reduced cell number and lineage marker expression ap-

peared. These results suggest that there are common mechanisms in the regulation of cell-cycle between ES cells and embryo. To reveal the cell-cycle regulation mechanism by Klf, we amplified the cDNA from embryos and performed the microarray analysis. Then we found that FGFR signaling pathway related genes was hyperactivated in Klf5 KO embryos. Pharmacological inhibition for this pathway by specific inhibitor resulted in significant rescue for Klf5 KO phenotypes. Interestingly, Nanog positive pluripotent cells were emerged in ICM of Klf5 KO embryos after pharmacological inhibiton of FGFR signaling pathway. We examined whether Klf5 KO ES cells are derived from rescued blastocyst. We obtained 60 ES cell lines from 92 embryos, and remarkably, 7 lines of Klf5 KO ES cell were derived from rescued embryos. These results clearly shows that FGFR signaling pathway is hyperactivated in Klf5 KO embryos. Since our previous study revealed that Klf5 KO ES cells showed upregulation of Fgf5 expression, we examined whether FGFR signaling pathway is hyperactivated in Klf5 KO ES cells. Compared to WT ES cells, Klf5 KO ES cells exhibited elevated levels of the phosphorylation of ERK1/2. Moreover, when Klf5 KO ES cells were cultured in the presence of Mek inhibitor or Fgfr inhibitor, defective proliferation was partially, but significantly rescued. These results suggest that Klf5 suppress FGFR signaling pathway in the embryo and ES cells, and act as a safeguard for the maintenance of pluripotent stem cells.

W-2297

MAINTENANCE OF HUMAN EMBRYONIC STEM CELLS IN FEEDER AND bFGF FREE CULTURE SYSTEM USING CONDITIONED MEDIA FROM IMMORTALIZED HUMAN FORESKIN FIBROBLAST CELLS

Sujata Mohanty[1], Pooja Teotia[1], Balram Airan[2]

[A]Stem Cell Facility, [B]Department of Cardiothoracic and vascular Surgery, [1]AIIMS, New Delhi, New Delhi, India

Mouse and Human feeder cells have important role in the maintenance of Human Embryonic Stem Cells (hESCs) in undifferentiated state as well as for their propagation. However, use of feeder cells in hESC culture limits their future therapeutic applications. There have been reports of using conditioned media (CM) derived from mouse or human feeder cells for the propagation of hESC in feeder free culture. These CM contain certain factors required for maintaining ESC in undifferentiated and pluripotent state. Recently, TGF-β and IGF-II have been shown to be important for the maintenance of hESC.

Feki et. al. reported the establishment of stably Immortalized Human Foreskin Fibroblasts (I-HFF) cells to be used as feeders for culturing hESC and iPSC. These cell lines have been stably transduced with secreted form of bFGF. This study aims to (i) quantiate the secretion of TGF- β and IGF-II by I-HFF feeder, (ii) explore the potential of CM derived from I-HFF feeder to support ES cells in feeder free system, (iii) minimize the dose of an exogenous bFGF required to sustain hESC in feeder free system.

The secretion of TGF- β and IGF-II from the mitomycin-C treated I-HFF supplemented with various exogenous bFGF concentration (0, 2, 5 and 10ng/ml) was assessed by ELISA. The KIND -1 hES cell lines were gradually adapted to grow in feeder free system on geltrex coated culture dishes using CM at varying concentration of exogenous bFGF supplementation (0,2,5 and 10ng/ml). The KIND-1 cell line cultured in hESC media with 10 ng/ml bFGF was taken as positive control. The hESC line grown in feeder free culture with CM was assessed on the basis of morphology, expression of pluripotent makers at 1st, 3rd, 5th and 10th passage by Immunoflorescence and flow cytometry. Any karyotypic abnormalities were also assessed at 20th passage.

ELISA results confirmed that the level of both TGF- β and IGF-II secretion was comparable at all bFGF treated group versus no exogenous bFGF added. The cells cultured in the CM in the feeder free conditions even after 20 passages, showed typical hESC morphology and expression of pluripotency-related proteins, SSEA-4, TRA-1-60, OCT4, alkaline phosphatase and normal karyotypes in all groups compared to positive control. Flow cytometric analysis for TRA1-60 and SSEA-4 surface marker expression shows the increasing trend but the difference was negligible among different groups. (From 0-, 2, 5,10ng/ml and positive control TRA1-60: 0- 75.6% + 3.86, 2- 76.87% + 5.64, 5- 77.28% + 5.21, 10- 78.1% + 5.83 and 81.6% + 3.53, SSEA-4: 0- 81.47%+4.27, 2- 82.9%+3.86, 5- 82.73%+3.80, 10- 84.07%+5.72 and 82.73%+3.80 respectively). There was no difference in the expression of pluripotency-related genes (OCT4, SOX-2, c-MYC, Klf-4 and NANOG) in test groups as compared to positive control as reveled by semi quantitative RT-PCR.

In conclusion, (i) the I-HFF CM secrete TGF- β and IGF-II without addition of exogenous bFGF, (ii) addition of various dose of bFGF did not scale up the secretion of TGF- β and IGF-II, suggesting that the endogenous bFGF secretion is sufficent to maintain auto regulation of TGF- β and IGF-II, (iii) the I-HFF CM is able to support and maintain hESC propagation and pluripotency without addition of exogenous bFGF. Thus the CM is an alternative to achieve cost effective and feeder free culture system.
Acknowledgment: We thank Prof. Anis Feki, Geneva University, Switzerland and Dr. Deepa Bhartia, National Institute for Research in Reproductive Health, India for providing I-HFF and KIND-1 hESC lines.

W-2298

ACTIVATION OF DNA DAMAGE RESPONSE DOES NOT INDUCE G1/S CHECKPOINT IN MOUSE EMBRYONIC STEM CELLS

Irina I. Suvorova, Valery A. Pospelov
Laboratory of Molecular Mechanisms of Cell Differentiation, Institute of Cytology, St-Petersburg, Russian Federation

The DNA damage response (DDR) integrates different signaling pathways activated by DNA-damaging agents and is primarily coordinated by ATM and ATR kinases, initiating a cascade of phosphorylation events, eventually resulting in cell cycle checkpoint activation, DNA repair and apoptosis. Mouse embryonic stem cells (mESCs) are characterized by high proliferation activity and do not undergo G1/S checkpoint upon DNA-damage. In this study we examined the ATM/ATR signaling pathway to analyze DNA repair capacity of mESCs and checked the functionality of the p53-p21/Waf1 pathway that provides G1/S checkpoint in somatic cells.
According to flow cytometry analysis, inhibitors of ATM, ATR, and Chk1 but not Chk2 affect cell cycle both in control and irradiated (IR) mESCs. ATM+ATR inhibitor caused accumulation of G1-phase cells and decreased the amount of S phase cells. Treatment of mESCs with ATM/ATR inhibitor enhanced the effect of IR on cell cycle: it further reduced the number of cells in S phase and caused the accumulation of cells in G2/M. Inhibition of ATM kinase caused a weaker effect. We next suppressed ATM/ATR targets, Chk1 and Chk2, and found that inhibition of Chk1 in IR-treated mESCs caused accumulation of mESCs in G1 phase and decrease of G2 cells.
MTT test revealed the ATM, ATR, Chk1, Chk2 contribution to survival of mESCs after DNA damage. Treatment with ATM/ATR inhibitor increased cell death in control and irradiated mESCs, whereas ATM inhibitor caused no effect. Again, inhibition of Chk1 in IR-treated cells caused an effect compared with ATM/ATR inhibitor + IR. Chk2 inhibitor had no effect on the survival of mESCs.
Immunofluorescent confocal microscopy and western blotting for phospho-ATM (Ser-1981) and phospho-ATR (ser-428) showed that in mESCs ATM/ATR kinases are phosphorylated and accumulated in the nuclei after DNA damage. We also showed that irradiated mESCs form H2AX foci that co-localize with 53BP1 protein and Rad51. In control, non-irradiated mESCs, uninduced γH2AX foci do not contain 53BP1.
By using antibodies against phospho-p53 (Ser-15) and acetyl-p53 (Lys-379), we showed that IR or H_2O_2 induced the p53 stabilization and its transcriptional activity. However, although p21 mRNA is clearly up-regulated after DNA damage, p21 protein is not detectable. To investigate the mechanisms of negative regulation of p21, we used proteasome inhibitors (lactacystin, MG132) and inhibitor of histone-deacetylases sodium butyrate (NaBut). Both NaBut and proteasome inhibitors cause the accumulation of p21Waf1 implying the regulation of p21Waf1 as at the level of gene regulation as at the level of protein stability.
The findings suggest that despite the absence of a functional G1 checkpoint caused by dysfunction p53/p21Waf1 pathway in mESC, the ATM/ATR signaling is activated with comcomitant activation of downstream targets that is a necessary step to initiate the process of DNA repair.

W-2301

MOLECULAR MECHANISMS FOR BUFFERING PLURIPOTENCY IN EMBRYONIC STEM CELLS

Dmitri Papatsenko[1], Ihor R. Lemischka[2], Henia Darr[2]

[1]Icahn School of Medicine at Mount Sinai, New York, NY, United States, [B]Department of Developmental & Regenerative Biology, [1]Icahn School of Medicine at Mount Sinai, New York, NY, United States

Until recently, knowledge regarding hierarchical organization of pluripotency network in mESC was relatively fuzzy. Most studies assume the top (or central) position of the Oct4-Sox2-Nanog circuit, while the exact connections between the factors remain unknown.
Based on the analysis of knock-down studies of the core pluripotency factors (differentiation experiments) we proposed a new view of the network relevant to pluripotency, in which Oct4 occupies the top position in the hierarchy and Nanog, Esrrb, Sox2 occupy lower level. The hierarchical analysis revealed a new network motif, incoherent feed-forward loop Oct4-Nanog-Sall4, which appears to play an important role in pluripotency.
Recently published data (Cell. 2012, 150(6):1209-22) suggested presence of regulatory link Sall4 -> Oct4, thus adding a feedback connection to the identified Oct4-Nanog-Sall4 network motif.
Quantitative exploration of solutions for the identified network connecting the three genes, along with new hierarchical structure of the pluripotency network suggested a molecular mechanism for safeguarding or buffering of pluripotency. Available experimental data supports the central position of the Oct4-Nanog-Sall4 in the pluripotency network and the proposed buffering role of the identified network motif.

W-2303

HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN A2/B1 REGULATES THE SELF-RENEWAL AND PLURIPOTENCY OF HUMAN EMBRYONIC STEM CELLS VIA THE CONTROL OF THE G1/S TRANSITION

Hong Seo Choi[1], HYUN MIN LEE[1], YOUNG-JOO JANG[2], CHEORL-HO KIM[3], CHUN JEIH RYU[1]
[1]Sejong unversity, Seoul, Korea, Republic of, [2]Dankook unversity, Cheonan, Korea, Republic of, [3]Sungkyunkwan university, Suwon, Korea, Republic of

Self-renewal and pluripotency of human embryonic stem
cells (hESCs) are a complex biological process for maintaining hESC stemness.
However, the molecular mechanisms underlying these special properties of hESCs
are not fully understood. Heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNP
A2/B1) is a multifunctional RNA-binding protein whose expression is related to cell proliferation and carcinogenesis. In this
study, we found that hnRNP A2/B1 expression was localized to undifferentiated
hESCs, and decreased upon differentiation of hESCs. hnRNP A2/B1 knockdown
reduced the number of alkaline phosphatase-positive colonies in hESCs and led
to a decrease in the expression of pluripotency-associated transcription
factors OCT4, NANOG and SOX2, indicating that hnRNP A2/B1 is essential for hESC
self-renewal and pluripotency. hnRNP A2/B1 knockdown increased the expression
of gene markers associated with the early development of three germ layers, and
promoted the process of epithelial-mesenchymal transition, suggesting that
hnRNP A2/B1 is required for maintaining the undifferentiated and epithelial
phenotypes of hESCs. hnRNP A2/B1 knockdown inhibited hESC proliferation and
induced cell cycle arrest in the G0/G1 phase before differentiation via
degradation of cyclin D1, cyclin E, and Cdc25A. hnRNP A2/B1 knockdown increased
p27 expression and induced phosphorylation of p53 and Chk1, suggesting that
hnRNP A2/B1 also regulates the G1/S transition of hESC cell cycle through the
control of p27 expression and p53 and Chk1 activity. Analysis of signaling
molecules further revealed that hnRNP A2/B1 regulated hESC proliferation in a
PI3K/Akt-dependent manner. These
findings provide for the first time mechanistic insights into how hnRNP A2/B1
regulates hESC self-renewal and pluripotency.

W-2304

TCF-3 AND JNK IN THE GLUCOSE MEDIATED CONTROL OF PLURIPOTENCY AND DIFFERENTIATION IN MURINE ESCS

Darcie L. McClelland Descalzo[1], Tiffany Satoorian[1], Devon D. Ehnes-Zepeda[1], Roger J. Davis[2], Bradley J. Merrill[3], Nicole I. zur Nieden[1]

[1]Cell Biology and Neuroscience, University of California, Riverside, Riverside, CA, United States, [2]Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, United States, [3]Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago, IL, United States

Current studies estimate that 10-18% of women of reproductive age have diabetes or a related disorder. This is alarming considering that 7-13% of diabetic mothers give birth to a child with a major structural birth defect. Though recent advances in insulin therapy have significantly curtailed miscarriages due to hyperglycemia during pregnancy, glucose induced defects suffered by the fetus during the pre-implantation stage of development still pose a huge problem to modern medicine. While scientists have isolated the elevated maternal blood glucose levels as the primary cause of fetal malformations, the mechanisms by which glucose stress induces birth defects still remain to be elucidated.

Our lab utilizes mouse embryonic stem cells as a model for pre-implantation embryos. By exposing cells to different glucose concentrations and tracking changes in characteristics related to the pluripotent state, we aim to gain insights into what may be happening to human embryos when the basic plan for life is laid out. In addition, we examine the role of β-catenin (CatnB) to determine how its levels and localization adapt to fluctuations in glucose levels. Recently, we have been examining the contributions of TCF-1, TCF-3, and JNK to CatnB transcriptional activity and thus embryo development in different glucose concentrations. When wildtype cells are exposed to hyperglycemic conditions, they lose the ability to remain pluripotent. In addition, cells increase nuclear TCF-3 protein and decrease nuclear TCF-1 protein. Because TCF-3 has previously been shown to inhibit pluripotency factors and is increased in response to glucose, we hypothesized that it may contribute to the phenotypic changes associated with hyperglycemia.

Cells with a genetic deletion of TCF-3 maintain pluripotency and show increased mRNA levels of Oct-4 and Nanog in all glucose concentrations when compared to wildtype cells. From this data, we determined that TCF-3 may affect the switch from pluripotency to differentiation in hyperglycemia. In addition, we performed sequential Chromatin Immunoprecipitation and found that the amount of TCF-3 bound to the promoters of Oct4 and Nanog is increased upon glucose challenge while the amount of TCF-1/CatnB bound to these promoters is simultaneously decreased. This data suggests that TCF-1/CatnB and TCF-3 may compete for binding on target gene promoters and that glucose may impact the ratio of binding, ultimately influencing the pluripotency state of the cells.

Upstream, TCF-3 nuclear shuttling may be regulated by c-jun NH(2)-terminal kinase (JNK). Thus, JNK may indirectly contribute to the phenotypic switch to differentiation through increasing nuclear TCF-3. Indeed, JNK activation increases with glucose levels and this correlates with an increase in nuclear TCF-3 protein. When cells were treated with a JNK inhibitor, protein levels of TCF3 were decreased and levels of Nanog, which is known to be inhibited by TCF-3, were increased. Currently, we are examining stem cell fate in cells with a genetic deletion of JNK to determine whether its absence will deplete nuclear TCF-3 and lead to increased pluripotency. Taken together, these results will bring us closer to understanding the mechanisms behind phenotypic changes in unspecified cells in the early embryo. Once we can fully elucidate this mechanism, it will provide valuable knowledge for future studies on preventing birth defects in children born to diabetic mothers.

W-2305

DOWNREGULATION OF OCT-4 INHIBITS PROLIFERATION OF ES CELLS BY INDUCING G1 CELL CYCLE ARREST

Jungwoon Lee, Inyoung Kang, **Jungho Kim**
Sogang Univ, Seoul, Korea, Republic of

Mouse and human embryonic stem (ES) cells display unusual proliferative properties and can produce pluripotent stem cells indefinitely. Both processes might be important for maintaining the stemness of ES cells; however, little is known about how the cell cycle fate is regulated in ES cells. Oct-4, a master switch of pluripotency, plays an important role in maintaining the pluripotent state of embryonic stem cells and may prevent the expression of genes activated during differentiation. Using ZHBTc4 ES cells, we have investigated the effect of Oct-4 on ES cell cycle control, and we found that Oct-4 down-regulation in ES cells inhibits proliferation by blocking cell-cycle progression in G0/G1. Deletion analysis of the functional domains of Oct-4 indicates that the overall integrity of the Oct-4 functional domains is important for the stimulation of S-phase entry. We also show here that the p21 gene is a target for Oct-4 repression. Furthermore, p21 protein levels were repressed by Oct-4 and were induced by the down-regulation of Oct-4 in ZHBTc4 ES cells. Therefore, the down-regulation of p21 by Oct-4 may contribute to the maintenance of ES cell proliferation.

W-2306

CONVERSION OF THE PLURIPOTENT STATE IN NON-HUMAN PRIMATE ES CELLS

Seiji Shiozawa[1], Junko Okahara[2], Hirotaka James Okano[3], Toshiaki Noce[1], Erika Sasaki[1], Hideyuki Okano[1]
[1]Keio University School of Medicine, Tokyo, Japan, [2]Central Institute for Experimental Animals, Kanagawa, Japan, [3]Jikei University School of Medicine, Tokyo, Japan

Although both primate and mouse embryonic stem (ES) cells are pluripotent stem cell lines derived from inner cell mass (ICM) of blastocyst stage embryos, they show distinct characteristics. Currently, primate ES cells have been considered as counterpart of mouse epiblast stem cells (EpiSCs) because of their close resemblance. The pluripotent state of mouse EpiSCs is termed primed state to distinguish from naïve state pluripotency of mouse ES cells. Primate ES cells have been regarded as primed state pluripotent stem cells and the naïve state pluripotent stem cells in primate has not been established so far. Using ES cells of common marmoset, a small non-human primate, we have carried out the conversion from primed state to naïve state pluripotency.
Doxycycline-inducible (DOX-inducible) reprogramming factors transgenes were introduced into common marmoset ES cells by piggyBac transposon transgenesis, and the cells were cultured in a medium containing LIF, Dox and cocktail of small molecules. Resulting cells formed mouse ES cell-like dome-shaped colonies and maintained expression of pluripotency markers. Furthermore, these cells showed similar gene expression pattern to mouse ES cells and ICM cells of marmoset blastocyst. These phenotypes were lost by addition of JAK inhibitor to culture, suggesting maintenance of this pluripotent state require LIF/STAT3 signal.
These properties of the common marmoset ES cells closely resembled to mouse ES cell, suggesting that the cells acquired naïve state pluripotency. To further confirm naïve pluripotency of the cells, we are currently carrying out chimera formation assay.

W-2307

HESC EXPANSION AND STEMNESS ARE INDEPENDENT OF GJIC THROUGH CX43 BETWEEN HESC AND FEEDER CELLS

Jin-Su Kim[1], Seung-Twang Hwang[1], Daekee Kwon[1], Dong-Ryul Lee[1], Won Kim[2], Myung-Kwan Han[2], Soo-Hong Lee[1]
[1]CHA Univ, Seoul, Korea, Republic of, [2]Chonbuk National Univ, Gyeonggi-do, Korea, Republic of

Human embryonic stem cells (hESCs) are a promising and powerful source of cells for applications in regenerative medicine, tissue engineering, cell-based therapies, and drug discovery. Many researchers have employed conventional culture techniques using feeder cells to expand hESCs in significant numbers, although feeder-free culture techniques have recently been developed. In regard to stem cell expansion, gap junctional intercellular communication (GJIC) is thought to play an important role in hESC survival and differentiation. Indeed, it has been reported that hESC-hESC communication through connexin 43 (Cx43, one of the major gap junctional proteins) is crucial for the maintenance of hESC stemness during expansion. However, the role of GJIC between hESCs and feeder cells is unclear and has not yet been reported. This study therefore examined

whether a direct Cx43-mediated interaction between hESCs and human adipose-derived stem cells (hASCs) influences the maintenance of hESC stemness. Over 10 passages, hESCs cultured on a layer of Cx43-downregulated hASC feeder cells showed normal morphology, proliferation (colony growth), and stemness, as assessed by alkaline phosphatase (AP), Oct4, Sox2, and Nanog expression. These results demonstrate that Cx43-mediated GJIC between hESCs and hASC feeder cells is not an important factor for the conservation of hESC stemness and expansion.

W-2308

A SERUM-FREE AND FEEDER-FREE DEFINED MEDIUM THAT ENABLES WEEKEND-FREE CULTURE OF UNDIFFERENTIATED HUMAN PLURIPOTENT STEM CELLS

Min Lu[1], Naomi Guyette[1], Boris Greber[2], Vi Chu[1]

[1]Bioscience Division, Stem Cell Group, EMD Millipore, Temecula, CA, United States, [2]Max Planck Institute for Molecular Biomedicine, Münster, Germany

Current media formulations used to culture undifferentiated pluripotent human ES and induced pluripotent stem (iPS) cells require media replenishment every day and at least 1 media exchange on the weekend. The costs in media reagents and human resources become prohibitively expensive as increasing numbers of core labs and consortiums are focused on generating diseased human iPS cell models. We have identified 11 essential components in a serum-free and feeder-free medium that enables weekend-free culture of undifferentiated human pluripotent stem cells and allows for media exchanges every other day without compromising the morphology or long term functionality of pluripotent stem cells. These 11 components include HSA, lowered FGF (10% of E8), TGFb1 (25% of E8) and Activin A concentrations and small molecule inhibitors to suppress spontaneous differentiation.
For the study, H9 human ES cells and 2 different clones of transgene-free human iPSCs were used. Transgene-free human iPSCs were generated by a Cre-excisable polycistronic lentiviral vector expressing the “stem cell cassette” (STEMCCA) comprised of all four transcription factors (OKSM) followed by exposure of the full reprogrammed iPSC to cell permeable TAT-Cre recombinant protein. All three pluripotent cell lines whether originally cultured in feeder-based or feeder-free cultures could be easily and directly adapted to the 11-component medium without noticeable differences in cell vitality and cell health. Pluripotent cells maintained in the 11 component medium (>20 passages) expressed high levels (>90%) of pluripotency markers including SSEA4, OCT4, TRA-1-60 and TRA-1-81; possessed normal karyotype; and were capable of differentiating into derivatives of all three germ layers in vitro and in vivo.
Remarkably, H9 cells remained undifferentiated and exhibited minimal signs of spontaneous differentiation and could furthermore be serially passaged with a minimal 1 day per week media exchange. While a 25-33% reduction in the number of attached colonies was observed, the colonies remained undifferentiated and proliferated at a similar apparent rate (i.e. 5-6 days between passages) to controlled cultures of regular media exchanges. These unexpected results led us to determine the long-term effects of a more moderate feeding regiment of every other day combined with an absence of weekend media replacement. Pluripotent cells maintained in this moderate feeding regiment exhibited high cell health with minimal spontaneous differentiation; expressed high levels of pluripotency markers and possessed a normal karyotype. Current efforts are aimed at identifying the critical component(s) that confer this advantage and whether nutrient-sensitive signaling pathways that regulate energy metabolism may be involved.

W-2311

THE RABBIT PRE IMPLANTATION EMBRYO AS A PARADIGM TO EXPLORE NAIVE EMBRYONIC STEM CELL DERIVATION IN NON RODENT SPECIES

Pierre OSTEIL[1], Yann TAPPONNIER[1], Anaïs MOULIN[1], Thierry JOLY[2], Pierre SAVATIER[1], Marielle AFANASSIEFF[1]

[1]Stem cell and Brain Research Institute, INSERM U846, INRA USC1361, BRON, France, [2]VetAgroSup, UPSP ICE, ISARA-Lyon, Lyon, France

The scarcity of pre-implantation embryos in human is a major limitation to the improvement of embryonic stem cell (ESC) derivation in non-rodent species. Rabbit is a good alternative as it can produce up to 30 embryos per super-ovulated females. Moreover, all rabbit ESC line produced so far showed the characteristics of primed pluripotency like human ESCs. In this work, we investigated the conditions suitable for the generation of naïve ESCs in rabbits. A first experiment aimed to study the effect of the pharmacological inhibitor of MEK signaling, PD0325901, on embryo development and ESC line derivation. We collected 576 eight-cell stage embryos, of which 317 were cultured in the presence of MEK inhibitor until they reached the early blastocyst stage. No difference in the rate and quality of embryo development between MEK inhibitor-treated and control embryos was observed. ICMs were isolated by immunosurgery and plated onto gelatin- or fibronectin-coated dishes in various media (DMEM/F12 + 20% KOSR, N2B27) supplemented with GSK3 inhibitor (CHIR99021), MEK inhibitor, and LIF (2i/LIF), or not. 80% of the ICMs plated, but none could be expanded beyond passage 2. We concluded that inhibition of MEK signaling fails to prevent spontaneous differentiation of pluripotent stem cells in rabbit. A second experiment aimed to study the effect of LIF on ESC line derivation. We collected 262 ICMs, which were plated onto growth-inactivated mouse embryonic fibroblasts in DMEM/F12 supplemented with 20% KOSR (72), 20% KOSR + LIF (90), or 10% KOSR + 10% FCS + LIF (80). No outgrowth cultured in media lacking LIF could be expanded beyond passage 4. By contrast, 7 ESC lines were derived from outgrowths cultured and expanded in the presence of LIF, 2 in 20% KOSR + LIF, and 5 in 10% KOSR + 10% FCS + LIF. Two lines, designated rbES-LIF1 and rbES-LIF2, were expanded by gentle dissociation with collagenase until passage 40, and showed a normal karyotype. RbES-LIF1 and rbES-LIF2 displayed the cardinal features of pluripotent stem cells, i.e. expression of pluripotency markers, differentiation into derivatives of the 3 germ layers, and teratoma formation. However, they could not be cultured onto gelatin-coated dishes, they did not express markers associated with naïve pluripotency in rodents, and they did not survive in 2i/LIF medium. We concluded that LIF facilitates the derivation of ESCs but does not support naïve pluripotency in rabbits. When rbES-LIF1 and rbES-LIF2 cells were propagated for 20 passages in 10% KOSR + 10% FCS + LIF, and were enzymatically dissociated with Accutase into single cell suspensions, they acquired chromosomal abnormalities (43XX; 45XY). The same observation was made with the 5 freshly derived ESC lines in 10% KOSR + 10% FCS + LIF with Accutase, of which 3 displayed abnormal chromosome numbers. We concluded that LIF-dependent rabbit ESCs cannot be propagated under stringent conditions without frequent chromosomal rearrangement-based adaptation.

W-2312

INCREASED DLK-1 EXPRESSION IN HUMAN EMBRYONIC STEM CELLS, HAS A PUTATIVE ROLE IN THE MAINTAINANCE OF PLURIPOTENCY.

David J. Harley, Dylan Stavish, Paul J. Gokhale, Peter W. Andrews
Department of Biomedical Science, University of Sheffield, Sheffield, United Kingdom

Delta-like homolog 1 (DLK-1) is a trans-membrane domain cell signalling protein that interacts with NOTCH. It is known to be up-regulated when comparing normal and culture adapted human embryonic stem (hES) cells. Culture adapted hES cells exhibit an increased capacity to self renew, and by profiling normal and adapted against one another we have highlighted genes important for hES maintenance. DLK-1 has been indicated in many tissue specific areas and associated cancerous phenotypes but the mechanisms by which signalling occurs is not fully characterised. Moreover it appears that DLK-1 can act in an equivalent manner to NOTCH and its counterparts, via cell-cell interactions, peptide cleaving micro-environmental effects and as a receptor itself, with each of these designating a specific downstream effect. NOTCH signalling is known to regulate the hierarchical differentiation in many cell populations within the adult and is essential for normal development.
Utilizing normal and adapted hES cells and their malignant counterparts, human embryonal carcinoma (hEC) cells, we investigated the role of DLK-1 in the adaptation of hES cells and its links with other pathways at the gene expression and proteomic level. This has highlighted a correlation between the ability of cells to self renew and an increase in DLK-1 expression. Furthermore, markers associated with pluripotency, differentiation and more specifically those involved in the NOTCH signalling pathway have also been identified. In addition we have explored the effects of DLK-1 signalling in the hES micro-environment and the mechanisms by which it increases the self-renewal and pluripotency of hES lines.

Knockdown of DLK-1 in the adapted hES cells triggers the onset of differentiation at an earlier stage in colony development, measured by the down regulation of pluripotency markers OCT-4, NANOG and SSEA3. NOTCH1 signalling is elevated on induction of the knockdown of DLK-1 and has been shown to induce the differentiation of hES cells leading to a reduction in self renewal and upregulation of markers of differentiation. Conversely hES cells over-expressing DLK-1 exhibit an increase in proliferation.

W-2313

SELF ASSEMBLING SCAFFOLD SUPPORTS PLURIPOTENT GROWTH OF EMBRYONIC STEM CELLS

Christina McKee, Ferman Chavez, Rasul Chaudhry
Oakland University, Rochester, MI, United States

Embryonic stem cells (ESCs) have unlimited self-renewal and differentiation potential. In vitro culturing of ESCs is laborious, technically challenging, and often leads to the loss of cell lines due to differentiation. To address these problems, considerable attention has been focused on devising culture conditions that mimics in vivo stem cell microenvironments. We hypothesized that three dimensional (3D) culture conditions may be better than routinely used 2D cultures for the maintenance and expansion of ESCs. The objective of this study was to test biodegradable nanomaterials to produce a 3D culture matrix capable of long-term propagation of pluripotent ESCs. To achieve 3D culture conditions, self-assembling scaffolds were developed by mixing thiol-functionalized dextran (Dex-SH) and poly (ethylene glycol) tetra-acrylate (PEG-4-Acr) via Michael addition reaction. Testing of various parameters including the components of scaffold and encapsulation of ESCs led to the development of 3D culture conditions that promoted the self-renewal and pluripotent growth of ESCs for an extended period of time. The scaffold grown cells exhibited typical morphological and biochemical characteristics similar to ESCs grown in 2D culture. Quantitative RT-PCR analysis showed that 3D cultured ESCs expressed a pluripotent gene, Klf4 at a level comparable to the cells grown in traditional 2D culture. Interestingly however, two prominent pluripotent genes, Oct-4 and Nanog, were expressed up to 3 fold higher levels and remained high until the scaffolds started to degrade after 10 to 40 days. 3D scaffold grown ESCs maintained their pluripotency as they readily differentiated into adipogenic, myogenic, neural, osteogenic and chondrogenic cell lineages even after prolonged 3D culture. Overall, the self-assembling scaffold provided the necessary support and maintained undifferentiated growth of ESCs for several weeks without passaging. Our findings provide a reliable, reproducible, cost effective, and robust culturing system that should lead to increased applications of ESCs for understanding basic development and differentiation mechanisms, tissue engineering and cell therapy.

W-2314

HUMAN TUBE MESENCHYMAL STROMAL CELLS AS FEEDER-LAYERS FOR HUMAN EMBRYONIC STEM CELLS

Mayra Pelatti[1], Mariane Secco[1], Carlos Czeresnia[2], Paulo Perin[3], Tatiana Jazedje[4], Mayana Zatz[1]
[1]Institute of Bioscience, University of São Paulo, São Paulo, Brazil, [2]Célula Mater, São Paulo, Brazil, [3]CEERH Specialized Center for Human Reproduction, São Paulo, Brazil, [4]EPM UNIFESP, Federal University of São Paulo, São Paulo, Brazil

Cultivation of Human Embryonic Stem Cells (heSCs) requires feeder-layers or conditioned culture media. One of the main problems to circumvent aiming the potential therapeutic use of heSCs is animal contaminants in the culture media or the co-cultivation with animal cells. Among the various possible available feeder-layers, the Murine Embryonic Fibroblast (MEF) is still the most used, followed by fibroblasts from human skin. Aiming to test a xenofree heSCs culture method and trying to mimic the human embryo environment we have evaluated whether human tube Mesenchymal Stromal Cells (htMSCs), which are closely associated to the human embryo in early stage of development, provide a better support for heSCs growth, when compared to MEF and fibroblasts from human skin. For this study, three htMSCs lineages, which were characterized by cytometry and their differentiation potential in vitro were evaluated. Afterwards, the three htMSC lineages were used as feeder-layers for heSCs (kindly donated

by Douglas Melton) culture. As control feeder-layers, MEF and fibroblasts from human skin were used in the course of each experiment and all the experiments were performed in six well culture dishes.
Twenty four hours after the culture establishment (feeder-layers with heSCs), culture media was collected from each dish well and the expression of b-FGF (basic-Fibroblast Growth Factor) and TGF-β (Transforming Growth Factor) was analyzed by ELISA. Expression of signaling molecules WNT2, WNT4 and WNT8b in the heSCs cultures was analyzed by Western blot, and the expression of specific embryonic markers (Oct-4, Nanog, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81 and KLF4) was confirmed by immunofluorescence and RT-PCR. Compared with MEF, htMSC feeder-layers cultures produced more heSCs colonies as well as a larger number of heSC per colony. Compared with human skin fibroblasts, htMSC feeder-layers produced heSCs colonies with more homogenous morphology (more undifferentiated colonies) and with a larger diameter, although the number of heSCs per colony was similar. The amount of b-FGF secreted by htMSCS was higher than the b-FGF released by the MEF twenty four hour after the heSCs culture establishment but the TGF- β secreted by htMSCs and MEF were similar. All the heSCs grown on htMSCs expressed WNT2, WNT4, WNT8b and all the specific embryonic markers tested, as expected. Thus, these results show that the use of htMSCs as feeder-layer represent an efficient xenofree method for heSCs cultivation.

W-2316

COMBINATORIAL SCREENING OF GELATIN NANOFIBERS AS CELLULAR SCAFFOLDS TO EXPAND HPSCS FOR LONG-TERM PERIOD

Li Liu, Ken-ichiro Kamei, Yong Chen
iCeMS, Kyoto University, Kyoto, Japan

Human pluripotent stem cells (hPSCs), such as embryonic and induced pluripotent stem cells (hESCs and hiPSCs), hold a promise for applications in cell-based therapy, tissue engineering, drug screening/discovery and regenerative medicine, since hPSCs have their unique characteristics, such as unlimited self-renewal capability under proper conditions and differentiation capability into any kind of cells in a human body. Recently, there are some efforts to develop new hPSC culture substrates, such as recombinant proteins, synthetic polymers and synthetic peptides, however, they require huge cost and labor intensiveness for preparation. Thus, it necessitates establishing the new substrates for hPSC expansion for long-term periods in a robust fashion.
Recently, nanofiber technology has attracted many researchers due to its application in fundamental cell biology as well as tissue engineering. In comparison with a conventional flat substrate, a nanofiberous substrate allows controlling the bulk-porosity, the surface texture and the three-dimensional (3D) surface topology for cell adhesion at a nanometer scale, altering focal-adhesion signaling cascades and consequently, the stem cell fate decision. During the past three years, we have successfully developed a nanofibres substrate as cellular scaffolds for expansion of mouse embryonic stem cells.
Here, we developed a new technology of gelatin electrospun nanofibers as a cellular scaffold for hPSCs long-term culture. We established a combinatorial library to vary density, crosslink conditions and gelatin molecular weight to screen the optimal conditions of fabricating nanofibers. Our results show that standardized gelatin nanofibers could support self-renewal for over 20 passages. However, hPSC couldn't passage on a gelatin-coated and crosslink treated flat substrate. In addition, we evaluated the pluripotent status of hPSCs cultured on gelatin nanofibers by immunocytochemistry, flow cytometry, RT-PCR, embryoid and teratoma formation and fluorescent in situ hybridization assay. According to such multiple angles of evaluation, we concluded that the gelatin nanofibers were able to serve as a cellular scaffold for expanding hPSCs while maintaining their pluripotent status and normal karyotype even after long-term culture. Finally, in order to understand how extrinsic 3D topological nanofibres influenced intrinsic cues during hPSC self-renewal, qPCR was carried out for all members of the integrin family. We found that gelatin flat-coated substrate and gelatin-nanofiber substrate were differed in the expression levels in some integrin members.
In the future, our goal is to develop a three-dimensional architecture of a cellular scaffold for cell transplantation therapies.

W-2317

CO-REGULATION OF PLURIPOTENCY AND ENHANCED GENETIC INTEGRITY AT THE GENOMIC LEVEL

Daniel Cooper[1], Christi Walter[2], John R. McCarrey[1]

[1]Biology, The University of Texas at San Antonio, San Antonio, TX, United States, [2]Biology, University of Texas Health Science Center San Antonio, San Antonio, TX, United States

Pluripotent stem cells, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have been reported to carry fewer genetic abnormalities than differentiated cells. Cells often deal with gross genetic aberrations such as polyploidy or aneuploidy retrospectively by programmed cell death, but for point mutations prospective avoidance must be achieved by DNA repair mechanisms. To characterize the mechanisms involved in the maintenance of enhanced genetic integrity in pluripotent stem cells, we have mined 18 previously published transcriptome and 5 cistrome databases describing gene expression and transcription factor binding in mouse and human ESCs, iPSCs and differentiated cells (fibroblasts). We observed significant up-regulation of DNA repair genes in both human and mouse ESCs and iPSCs relative to differentiated fibroblasts with very similar patterns of DNA repair gene expression in ESCs and iPSCs within each species based on expression data that was common to at least 50% of databases examined for each cell type. Functional analysis of these differentially expressed DNA repair genes by Ingenuity Pathway Analysis (IPA) revealed that mismatch repair and double-strand break repair by homologous recombination are the two most up-regulated repair pathways in pluripotent cells. Human and mouse pluripotent cells also showed significant differential expression of cell death genes compared to fibroblasts, but in contrast to DNA repair gene expression patterns, this included similar numbers of up- and down-regulated genes in the pluripotent cell types. However, upon further analysis of the cell death genes differentially expressed in pluripotent cells using IPA, we found that genes specifically involved in intrinsically regulated pro-apoptotic activity are primarily up-regulated, and those involved in extrinsically regulated pro-apoptotic activity plus those involved in anti-apoptotic activity are down-regulated in pluripotent cells. Thus, we confirmed differential expression of genes consistent with the maintenance of enhanced genetic integrity in pluripotent cells.
We next examined the mechanistic relationship between pluripotency and enhanced maintenance of genetic integrity at the genomic level by mining published cistrome data bases describing binding of the pluripotency factors OCT-4, SOX-2 and NANOG to target genes in pluripotent cells. We found evidence for direct binding ('first-degree interactions') of pluripotency factors to several of the genetic integrity (DNA repair and cell death) genes that are differentially expressed in pluripotent cells. In addition, we found evidence of indirect ('second-degree') interactions between pluripotency factors and genetic integrity genes mediated by binding of pluripotency factors to genes encoding transcription factors that, in turn, bind to differentially expressed genetic integrity genes. Thus, we demonstrate extensive interactions between the pluripotency and genetic integrity gene networks at the genomic level in pluripotent cells, and suggest that differential regulation (predominantly up-regulation) of genetic integrity genes is a fundamental readout of pluripotency which leads to enhanced maintenance of genetic integrity in pluripotent cells relative to differentiated cells.

W-2318

AN IMPORTANT ROLE FOR CDK1 IN CELL CYCLE REGULATION, CHECKPOINT CONTROL AND APOPTOSIS IN HUMAN EMBRYOINNIC STEM CELLS (HESC) REVEALED BY RNA INTERFERENCE STUDIES

Irina Neganova
Institute of Genetic Medicine, Newcastle upon Tyne, United Kingdom

We have shown in our previous studies that members of the cyclin-dependent kinase (CDKs) family show cell cycle dependent expression and in particular, one member of this family, CDK2 plays an important role in G1 to S transition and checkpoint regulation, DNA damage response and maintenance of pluripotency in hESC. In this study we have focused on the role of a closely connected CDK family member, CDK1. Our study shows that downregulation of CDK1 by RNA interference results in accumulation of hESC at G2 -phase of the cell cycle, polyploidy and loss of hESC pluripotency. Downregulation of CDK1, also induced downregulation of CHK2 and altered the maintenance of G2 checkpoint arrest, despite having no effect on its initial activation via CHK1. In addition, reduced expression

of Survivin, BCL2 and BCL-XL was observed together with increased expression of cleaved PARP1, a typical marker of apoptosis, suggesting that CDK1 downregulation may render hESC more sensitive to apoptosis. Yet, the level of pro-apoptotic active form of BAX was reduced and there were no changes in Caspase 3 or 9 activation , indicating that while apoptotic processes are initiated, final commitment is impaired upon CDK1 downregulation. Together, these data indicate that CDK1 has multiple roles in cell cycle progression, maintenance of pluripotency and cytokinetic stability as well as apoptosis execution.

W-2321

CHARACTERIZATION OF SEVEN HUMAN EMBRYONIC STEM CELL LINES (SNUHES32, 33, 34, 35, 36, 37 AND 38) DERIVED UNDER XENO-FREE CONDITIONS

Hye Won Seol[1], Jin Ah Baek[1], Juwon Jung[1], Bo Ae Yoon[1], Sun Kyung Oh[2], Hee Sun Kim[2], Seung-Yup Ku[2], Seok Hyun Kim[2], Shin Yong Moon[2], Young Min Choi[2]

[1]Institute of Reproductive Medicine and Population, Medical Research Center, Seoul National University, Seoul, Korea, Republic of, [2]Department of Obstetrics and Gynecology, Seoul National University, College of Medicine, Seoul, Korea, Republic of

INTRODUCTION: Human embryonic stem cells (hESCs) have potential as an unlimited source for biological research as well as treatment of incurable disease. One of the crucial requirements to enable to clinical use of these cells is to eliminate the risk of xeno-transmitted infections and immunoreactions caused by animal origin products in conventional cell culture system.
MATERIALS and METHODS: We were isolated inner cell mass of the
blastocyst from frozen embryos with mechanical dissection method. Isolated cells were expanded in gradual xeno-free culture conditions and established. Derived hESC lines were characterized by DNA fingerprinting, HLA typing, karyotype, AP staining, immunocytochemistry analysis for pluripotent cell surface markers (SSEA-3, SSEA-4, Tra-1-60, Tra-1-81, Oct-4), RT-PCR for undifferentiated gene expression (SOX2, Nanog, hTERT, Rex-1), mycoplasma detection test, in vitro differentiation capacity using embryoid body (EB) formation and Neu5Gc contamination examination by FACs.
RESULTS: Two hESC lines (SNUhES32 & SNUhES33) were established from phase 1 xeno-free culture condition (human foreskin fibroblasts (HFFs) cultured by IMDM basal medium containing FBS + xeno-free medium + humanized cell substrate) and five cell lines (SNUhES34, 35, 36, 37 & 38) were established from phase 2 (HFFs cultured by IMDM medium containing human serum (HS) + xeno-free medium + humanized cell substrate). All cell lines were identified by DNA fingerprinting, HLA typing and karyotype and were successful for freeze & thaw. Currently, these cell lines were succeeded in maintaining the self-renewing capacity and pluripotency of hESCs for more than 80 passages.
CONCLUSION: We have derived seven clinical-grade hESC lines under phase 1 and phase 2 xeno-free culture conditions. These hESC lines can be used for research but also clinical application in cell therapy.
This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MEST) (2012M3A9C6049722).

W-2322

MAINTENANCE OF HESC LINES USING HUMANIZED CULTURE CONDITION

Jin Ah Baek[1], Hye Won Seol[1], Juwon Jung[1], Bo Ae Yoon[1], Hee Sun Kim[2], Sun Kyung Oh[1], Seung Yup Ku[2], Shin Yong Moon[2], Young Min Choi[2]

[1]Institute of Reproductive Medicine and Population, Medical Research Center, Seoul National University, Seoul, Korea, Republic of, [2]Department of Obstetrics and Gynecology, Seoul National University, College of Medicine, Seoul, Korea, Republic of

INTRODUCTION: Human embryonic stem cells (hESCs) have the capacity to differentiate into all cell types, therefore holding the promise to be used in cell therapeutic applications. However, conventional culture conditions contain animal-derived components that bear the risk of transmitting non-human pathogens and incorporating non-human immunogenic molecules to hESCs. For these reasons, the research focus of hESC culture systems has been moving towards clinical applications. We report the development of a humanized culture condition without animal-derived material for clean-up using previously established hESC lines in this study.

METHODS: We set up the standardized culture condition using humanized materials, including CELLstart^{TM}, human foreskin fibroblast and a xeno-free medium containing knockOut^{TM} SR XenoFree (XF medium). After verifying the possibility of undifferentiated growth of SNUhES4 and H1 by replacing gelatin with CELLstart^{TM}, we used two different methods for maintaining cells in culture: sequential adaptation and direct adaptation. The former adapts in gradually increasing the ratios in the XF medium for the first four passages (25:75, 50:50, 75:25 and 100:0), while the latter directly uses a 100% XF medium.

RESULTS: For the early passages, both methods of humanized culture condition resulted in lower attachment rates and higher spontaneous differentiation rates, compared to conventional condition. Notably, the sequential adaptation method was more stable than the direct adaptation one. However, after further passages, both methods produced the same hESC characterizations as that of conventional culture condition, including morphology, growth behavior, expression of cell surface markers and stable karyotype. Finally, we observed that most of hESCs were negative for Neu5Gc (N-glycolyneuraminic acid, non-human immunogenic molecules) in humanized culture condition.

CONCLUSION: This study indicates that humanized culture condition of simplified compositions can be applicable to further culture optimization of hESC in a xeno-free medium without the loss of pluripotency and contamination of xenogenic sources. Clean-up of the hESC lines using this humanized culture condition would take one step closer to therapeutic applications. This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MEST) (2012M3A9C6049722)

W-2323

THE COMPARISON OF VARIOUS CONDITIONS AFFECTED ON SLOW-FREEZING OF HUMAN EMBRYONIC STEM

Jeoung Eun Lee, **Yuri Kim**, Myung Sun Shim, Sung-Geum Lee, Dong Ryul Lee
CHA Stem Cell Institute, CHA University, Seoul, Korea, Republic of

Successful cryopreservation of human Embryonic Stem Cells (hESCs) is able to preserve early passage stocks of hESCs and to facilitate worldwide distribution to researchers. There are two cryopreservation methods used for hESCs: vitrification and slow freezing with rapid thawing. Although several studies have reported 70~90% survival rates using various protocols, vitrification has several limitations preventing its widespread use such as the possibility of contaminating hESCs with infectious agents via contact with liquid nitrogen and very small volume of hESCs cryopreservation at once. Cryopreservation of hESCs using slow-freezing method might be suitable for large scale preservation but does not yet produce good results as much as in vitrification. Therefore, the optimization of several factors affected on hESC cryopreservation is needed. In this study, we tested the effect of DMSO concentration and the sodium ion in freezing medium on survival of hESCs after freezing and thawing in order to optimize the cryopreservation protocol of hESCs. CHA-hES15 cell line, established and maintained in CHA Stem Cell Institute, Korea, was used for this study. We used 0.25 straws as a storage vessel and a programmed cell freezer, and DMSO as a cryoprotectant for hESC slow-freezing. We collected clumps mechanically from hESC colonies for freezing and added freezing medium in two-steps. At first, we tested the effect of DMSO concentration on survival of hESCs after freezing and thawing; 10% DMSO+90% SR (A) and 5% DMSO+90% SR+5% DMEM/F12 (B). Then, we tested the effect of sodium ion in freezing medium; 10% DMSO+90% SR (C), 10% DMSO+20%SR+70% CJ2 (low sodium choline based medium) (D), and 10% DMSO+20%SR+70% M-CJ2 (modified CJ2 medium that sodium chloride replaces choline chloride) (E). We compared the colony forming rate of cryopreserved hESCs using various freezing media on fifth day after thawing. The Chi-square test was used for determining significant differences. The colony forming rates of group A, and C were 56.7% and 9.6%* (* P<0.001) respectively, in comparison of the DMSO con-

centration. The colony forming rates of group C, D and E for the effect of sodium ion on cryopreservation were 69.23%, 73.15% and 57.41%* (* P<0.05) respectively. The spontaneous differentiation rate (totally or ≥50% partially differentiated colony) was not significantly differentiated between all groups. From the results, the reduction of DMSO concentration (10% to 5%) in freezing medium was not appropriate for hESC cryopreservation, and the sodium ion in freezing media had a negative effect in survival of hESCs. Therefore, it is supposed that 10% DMSO, 20% SR and the rest of choline based medium (low sodium) might be an ideal freezing medium for efficient and cost effective hESC cryopreservation.

W-2324

ENDOPLASMIC RETICULUM PLAYS AN IMPORTANT ROLE IN THE REGULATION OF CALCIUM SIGNALING IN HUMAN EMBRYONIC STEM CELLS

Jijun Huang, Min Zhang, He Liang, Peng Zhang, Huang-Tian Yang
Key Laboratory of Stem Cell Biology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS) & Shanghai Jiao Tong University School of Medicine (SJTUSM), Shanghai, China

Human embryonic stem cells (hESCs), derived from the inner cell mass of blastocyst, have the ability to propagate indefinitely in culture while still remain the capacity to differentiate into all somatic cell types of three germ layers. Thus they are not only a potentially inexhaustible resource for the repair and restoration of organ functions but also a unique model for the study of human early development process. So far, much progress has been achieved on the understanding of regulatory mechanism of self-renewal and differentiation in hESCs, including the key transcription factors, signaling pathways and epigenetic regulators. However, little is known about the physiological properties and regulations of hESCs, including calcium (Ca^{2+}) signaling systems, while such knowledge would enrich our understanding of the characteristics of pluripotency, the regulation of self-renewal, such as the switch of cell cycle and the signaling response to extracellular stimuli, and the cell fate decision. Previous studies from other and our laboratories have demonstrated that Ca^{2+} signaling plays an important role in cell cycle regulation and cell lineage specification of mouse ESCs. In the present study, we aimed to (i) characterize Ca^{2+} signaling in hESCs; and (ii) elucidate the regulatory mechanism of Ca^{2+} signaling in hESCs. We therefore determined expression profiles of genes coding for major Ca^{2+} handling proteins in hESCs and then investigated the function of these proteins by using confocal Ca^{2+} imaging and flow-cytometry analysis, combined with pharmacological approaches and small RNA interference technique. When adenosine triphosphate was delivered to hESCs, which activates inositol-1,4,5-triphosphate receptors (IP_3Rs) in the endoplasmic reticulum (ER), a transient increase of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was detected. Such effect was partially inhibited by the pretreatment of the specific IP_3Rs antagonists, suggesting the involvement of IP_3Rs. Using an extracellular solution with high Ca^{2+} concentration, store operated Ca^{2+} entry (SOCE) was observed when intracellular Ca^{2+} stores were depleted by ER Ca^{2+}-ATPase blocker, and it was significantly changed after knock-down of IP_3Rs in hESCs. Inhibition of plasma membrane Ca^{2+} pump induced a large but slow increase of $[Ca^{2+}]_i$, while the Na^+/Ca^{2+} exchanger inhibitor induced a fast $[Ca^{2+}]_i$ elevation. These results indicate that hESCs already have a molecular basis to response extracellular stimuli by mobilizing a robust Ca^{2+} signaling regulatory cascade. Among it, the ER IP_3Rs-released Ca^{2+} plays an important role and store-operated Ca^{2+} channel mediated SOCE might be responsible for the Ca^{2+} refill after ER exhaustion.

W-2325

BCL2L1 IS THE KEY DRIVER OF 20Q11.21 GAINS IN HUMAN EMBRYONIC STEM CELLS

Ha T. Nguyen, Mieke Geens, Afroditi Mertzanidou, Kurt Jacobs, Karen Sermon, Claudia Spits
Reproduction and Genetics, Vrije Universiteit Brussel, Brussels, Belgium

BCL2L1 is the key driver of 20q11.21 gains in human embryonic stem cells

Ha T. Nguyen, Mieke Geens, Afroditi Mertzanidou, Kurt Jacobs, Karen Sermon, Claudia Spits
Research Group Reproduction and Genetics, Vrije Universiteit Brussel. Laarbeeklaan 101, 1090 Jette, Brussels.
Gain of 20q11.21 is a chromosomal abnormality that is recurrently found in human pluripotent stem cells and cancers, strongly suggesting that this mutation confers a selective advantage to these cells. In this work we studied three human embryonic stem cell (hESC) lines that acquired a 20q11.21 duplication during in vitro culture. Transcriptome analysis revealed that none of the genes located in the common region of duplication was consistently upregulated. On the other hand, the genes *CHCHD2* and *TRPC6*, located elsewhere in the genome, showed a strong and consistent change of expression in the mutant cells. These genes pointed towards the RhoA activation pathway, which in hESC is activated upon loss of cell-to-cell contact. This pathway lays upstream of *BCL2L1*, one of the genes located in the common region of duplication. We found that although the mRNA levels of Bcl-xL (the anti-apoptotic isoform of *BCL2L1*) were unchanged in mutant cells, the protein was 2-4 times more abundant. Furthermore, the phenotype of the mutant cells was consistent with Bcl-xL overexpression, with markedly decreased apoptosis after single-cell dissociation and increased clonogenic capacity. The uncoupling between the mRNA and protein levels of *BCL2L1* suggests a post-transcriptional regulation by another factor located on the common region of duplication. We investigated *ID1* as a candidate regulator, but found no increased levels of the ID1 protein, nor activation of the NF-kB pathway in the mutant cells, which would be required for post-transcriptional regulation of *BCL2L1*, ruling out *ID1* as the post-transcriptional regulator of *BCL2L1*.

W-2326

EFFECTIVE CRYOPRESERVATION OF HUMAN EMBRYONIC STEM CELL USING DIMETHYLSULPHOXIDE AND HYDROXYETHYLSTARCH COMBINATION.

Maristela Delgado Orellana[1], Gil Cunha De Santis[1], Danielle Aparecida Rosa Magalhães[1], Everton de Brito Oliveira Costa[1], Vivianane de Cássia Oliveira[2], Karen de Lima Prata[1], Patrícia Vianna Bonini Palma[1], Kuruvilla Joseph Abraham[3], Aparecida Maria Fontes[4], Dimas Tadeu Covas[1]
[1]Cell Biology, Regional Blood Center of Ribeirão Preto, Ribeirão Preto-São Paulo, Brazil, [2]Dentistry Department, Dentistry School of Ribeirão Preto, University of São Paulo, Ribeirão Preto-São Paulo, Brazil, [3]Department of Genetics, Medical School of Ribeirão Preto, University of São Paulo, Ribeirão Preto-São Paulo, Brazil, [4]Department of Genetics, Medical School of Ribeirão Preto, University of São Paulo, Ribeirão Preto-São Paulo, Brazil

Human embryonic stem cells (hESCs) are candidates for
many applications in the areas of regenerative medicine, tissue engineering,
basic scientific research as well as pharmacology and toxicology. An effective
freezing-thawing technique is crucial for clinical application and developing hESCs
banking. The aim of this study was to evaluate the recovery rate of
freezing/thawing hESCs using a combination of dimethylsulphoxide (DMSO) and
hydroxyethylstarch (HES) diluted in serum replacement (SR) solution. The efficiency of this cryopreservation solution
was evaluated in two slow-freezing applications in mechanical freezer (-80^{0}C)
and in a programmable cryopreservation system. This study shows for the first
time the combination of two cryoprotective reagents composed of HES, DMSO in SR
solution to establish a new slow-freezing protocol with high recovery rates of
undifferentiated hESC. The use of 10%
DMSO + 20% HES + 70% SR medium combined with mechanical detachment protocol in programmable
and in mechanical freezer leads to efficient hESC cryopreservation. We showed
that the recovery rates of hESC colonies (in programmable and in mechanical
freezer, respectively) with 10% DMSO + 20% HES + 70% SR medium were higher than
with Defined-medium® (3.88 and 2.9 vs 1.05 and 1.07; p = 0.000006903 and p =
0.04322) and higher than with classical medium 10% DMSO + 20% SR +70% DMEM
(3,88 and 2.9 vs 0.5 and 0.51, p = 0.0005136 and p = 0.0004253). Furthermore, after

thawing hESCs showed typical morphological characteristics, were positive for expression pluripotent markers, could still differentiate into the 3 germ layers, and showed normal karyotype. This new protocol allows, using simple steps without dedicated instrumentation, an efficient and economical hESC cryopreservation, ideal for hESC banking.

W-2327

PRDM14 INTERACTS WITH NANOG IN HUMAN EMBRYONIC STEM CELLS

Yun Shen Winston Chan
Genome institute of Singapore, Singapore, Singapore

Human embryonic stem cells (ESC) defined by their ability to self-renew indefinitely and to give rise to all adult cell types, are potentially invaluable in the field of regenerative medicine. However, this expanded cell fate potency also proves to be a hurdle for precise control of human ESC differentiation for various applications. Without detailed knowledge on how this cellular plasticity is regulated, their full potential remains to be harnessed. PRDM14 was identified in a genome-wide RNAi screen as an important determinant of the human ESC identity. PRDM14 binding across the human genome highly overlaps with the core pluripotency factor, highlighting the importance of PRDM14 in the core regulatory network. To further evaluate the importance of PRDM14 in the pluripotency network, its interaction with the core pluripotency regulators was investigated. PRDM14 was found to interact with NANOG via biochemical assays. This interaction was also reflected in their binding activities at genomic loci near pluripotency associated genes. Importantly PRDM14 and NANOG works in synergy to enhance transcriptional activity, supporting that this interaction is functional for gene regulation in hESC.

W-2328

GENOME-WIDE ANALYSIS OF MRNA TARGETS AND PROTEIN PARTNERS OF PUMILIO 1 IN MAINTAINING HESC PLURIPOTENCY

Yinghong Ma
Yale Stem Cell Center, Yale University, new Haven, CT, United States

Genome-wide Analysis of mRNA Targets and Protein Partners of PUMILIO 1 in Maintaining hESC Pluripotency
Yinghong Ma1*, Xiao Huang1*, Lin Geng1, Katherine Uyhazi1, Min Cang 1,2, Linfeng Wu 3, Michael Snyder3, Caihong Qiu1 and Haifan Lin1 1. Yale Stem Cell Center, Cell Biology, Yale University School of Medicine,10 Amistad St, New Haven, CT 06510; 2. Inner Mongolia University, Huhtot, China; 3. Stanford University, Stanford, CA; *co-first author.
Human embryonic stem cells (hESCs) are capable of self-renewal and differentiation into all cell types in the human body, thus they hold great potential for understanding human development and for regenerative medicine. Research on the genetic regulation of hESC self-renewal and differentiation has been mostly focused on epigenetic and transcriptional regulation. Little is known about the posttranscriptional regulation at the levels of mRNA stability and translation efficiency, which are also crucial to hESC self-renewal and differentiation. The Drosophila pumilio (pum) gene serves as a translational regulator to modulate gene expression by binding to the 3' untranslated regions (3'UTR) of their mRNAs. Our group previously demonstrated that Pum protein is essential for germline stem cell maintenance in Drosophila. Here we show that both PUM proteins in humans, PUM1 and PUM2, are abundantly expressed in hESCs and are predominately localized in the cytoplasm. Using RIP-ChIP assay, we identified hundreds of mRNA targets of PUM1 involved in several regulatory pathways, including translational regulation and apoptotic response. These mRNAs contain the conserved Pum binding sites, called Nanos Response Element (NRE), in their 3'-UTR. PUM1 does not affect the levels of the majority of PUM1-target mRNAs, as indicated by PUM1 knockdown experiments. This is consistent with the known role of PUM proteins in regulating translation but not mRNA stability. However, some of the target mRNAs are either up- or down-regulated in PUM1 knockdown hESCs, suggesting a new function of PUM1 in regulating the half-life of some of its target mRNAs. In order to explore how

PUM1 regulates its target mRNAs, we identified PUM1-interacting proteins by co-immunoprecipitation followed with mass spectrometry analysis. We are in the process of confirming the interaction between PUM1 and its putative partners.

W-2331

LIF AND HORMONE-DEPENDENT STAT3 ACT COOPERATIVELY TO SUSTAIN SELF-RENEWAL OF HUMAN ES CELLS

Hongwei Chen, Fabrice Gonod, Claire Hamela, Pierre-Yves Bourillot, Pierre Savatier
Stem-cell and Brain Research Institute, INSERM U846, Bron, France

Human embryonic stem cells (hESCs) depend on FGF2 and Activin/Smad signaling to self-renew in the primed pluripotency state, as opposed to mouse embryonic stem cells (mESCs), which exploit LIF/Stat3 signaling to sustain naive pluripotency. It is not clear whether Stat3 can also sustain self-renewal of hESCs. To answer this question, we engineered both our newly derived hESC line Oscar and the H9 hESCs with STAT3-ER^{T2}, a mutant estrogen receptor fused to mouse STAT3, which can be stimulated specifically by tamoxifen. Expression of STAT3-ER^{T2} in the presence of tamoxifen activates STAT3 target genes and supports self-renewal of mESCs in the absence of LIF. By contrast, bFGF-dependent Oscar-STAT3-ER^{T2} cells (designated F-OS3) did not activate STAT3 target gene expression upon treatment with tamoxifen, and remained strictly dependent on bFGF for self-renewal in the undifferentiated state; However, treatment of F-OS3 cells with both 10,000 U/ml hLIF and 250 µM tamoxifen resulted in the activation of some, albeit not all, STAT3 target genes (*c-fos*, *junB*, *Icam1*, *Cyp1b1*, *Ier3*, *Klf5*, *Gbx2*, *Sp5*, *Socs2*, and *Socs3*). Upon propagation in the presence of both factors, the F-OS3 cells progressively lost their dependency on bFGF, and continued self-renewing in the undifferentiated state. This was evidenced by the formation of tight colonies expressing the alkaline phosphatase, the pluripotency-associated transcription factors Oct4, Nanog, Sox2, and SSEA4. The cells have been passaged up to 60 times while maintaining a normal karyotype (46, XY). They retained the capacity to differentiate into derivatives of the three embryonic germ layers both *in vitro* by formation of embryoid bodies, and *in vivo* by formation of teratomas. These new cells thus expressed the cardinal markers and properties of pluripotency. They were designated TL-OS3 (TL for tamoxifen + LIF-dependency). TL-OS3 cells were strictly dependent on both LIF and tamoxifen for differentiation blockade. Moreover, after LIF/tamoxifen withdrawal and bFGF supplementation, they could be reverted to bFGF-dependency (so-called R-OS3 cells). Similar results were obtained with the H9 hESC line. Whole transcriptome analysis with Affymetrix DNA arrays showed that F-OS3 and R-OS3 cells shared similar transcriptional profiles, whereas TL-OS3 cells had a distinct profile, characterized by the activation of STAT3 target genes. Moreover, the TL-OS3 cells became permissive to trypsinization into single cells and formed mouse ESC-like colonies when cultivated in a medium supplemented with GSK3 and MEK inhibitors (CHIR99021 and PD0325901, respectively). Furthermore, activation of a number of genes whose expression is associated with naive pluripotency in rodents was observed. Collectively, these results show that LIF and hormone-dependent Stat3, together with GSK3 and MEK inhibitors, can sustain the self-renewal of human ESC cells in a state close to naive pluripotency.

W-2332

THE ROLE OF LAMININ 511 IN HUMAN PLURIPOTENT STEM CELLS

Sanna Vuoristo, Diego Balboa Alonso, Jaan Palgi, Timo Tuuri, Timo Otonkoski
Biomedicum Stem Cell Center, University of Helsinki, Helsinki, Finland

Results from several laboratories have shown that the extracellular matrix protein laminin 511 (lm-511) is a suitable substrate for human pluripotent stem cell (hPSC) culture. We have previously shown that hPSC synthesize, secrete and deposit lm-511 and that the production of lm-511 is associated with undifferentiated hPSC cultures. However, hPSC colonies are heterogeneous; they contain the central undifferentiated hPSCs, the more peripheral “niche” cells and the differentiated hPSC derived feeder (hdf) like cells. Our aims were 1) to characterize the patterns and the relationship of the laminin alpha 5 (lm α5) and pluripotency and early differentiation marker expressions within

hPSC colonies by using single-cell qPCR and 2) to investigate the lm-511 secretion in hPSC cultures using analytical confocal microscopy.
In large hPSC colonies, lm α5 expression concentrates to the peripheral cells of the colony. The expression correlates inversely with that of OCT4 and positively with the expression of various differentiation related genes such as SOX17 and N-CADHERIN. In addition, hPSCs express a novel variant of the full-length lm α5. The novel variant RNA is significantly shorter than the full-length lm α5 chain and it is only expressed in the middle of the colonies. The expression of the known, full-length lm α5 chain and its variant correlate inversely.
Confocal microscopy analysis showed that the peripheral cells indeed secrete significantly more lm-511 as judged by several parameters. The peripheral cells contain more than twice the amount of lm-511 secretory vesicles than the middle cells and the vesicle size of the peripheral cells is nearly double of that in the middle cells. Therefore, hPSCs create a lm-511 middle-peripheral polarity in large colonies. Interestingly, soon after passaging lm 511 is homogenously present throughout the small, undifferentiated hPSC colonies, likely promoting cell adhesion and organization of the colonies.
Taken together, we show that hPSCs dynamically modify their lm-511 secretion. The small, undifferentiated colonies secrete lm-511 throughout the colonies, while expanded colonies create a middle-peripheral polarity. In large colonies the undifferentiated hPSCs express a truncated lm α5 chain whose function is currently not understood. Our findings provide fundamental evidence for lm 511 as an important component of the hPSC in vitro niche.

Embryonic Stem Cell Differentiation

W-2333

CARDIOMYOCYTE DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS WITH THE STEMDIFF™ CARDIOMYOCYTE KIT

Jennifer Antonchuk, Reena Sandhu, Raymond Kwok, Cassie Ho, Terry E. Thomas, Allen C. Eaves, Sharon A. Louis
Research & Development, STEMCELL Technologies Inc, Vancouver, BC, Canada

Human pluripotent stem cells (PSCs) and induced pluripotent stem cells (iPSCs) have the potential to differentiate to any somatic lineage, given that specific inductive and/or inhibitory factors are added appropriately. A goal of this approach is to generate normal and disease-specific cardiomyocytes on which to test potential therapeutic agents for both their effectiveness and their toxicity. To this end, we have developed a defined, serum-free, feeder-free cell culture system for the directed differentiation of iPS cells into beating cardiomyocytes. To induce and promote differentiation, our system uses a monolayer culture with the multistep addition of a series of defined factors in specialized media at specific time points. The iPS cell lines tested to date include WLS-4D1 and WLS-1C (provided by Dr. William Stanford, Ottawa, Canada) and A13700 (from Life Technologies). Prior to differentiation, these iPS cell lines were maintained in mTeSR™1 on BD Matrigel©-coated plates. To initiate differentiation, mTeSR™1 was removed and replaced with STEMdiff™ Cardiomyocyte Basal Medium together with the addition of STEMdiff™ Cardiomyocyte Supplements A to D in a specific sequence over a period of 15 days. Synchronous beating was often visible in patches by day 12 and a synchronously beating cell sheet covering the whole well was formed by day 15. Cells from the indicated iPS cell lines were harvested on day 15 and analyzed by flow cytometry for expression of the intracellular cardiomyocyte marker cardiac troponin T (cTnT). Using this system, differentiated cells derived from WLS-4D1, WLS-1C and A13700 iPS cells expressed cTnT at a frequency of 44% (±27, n=10); 36% (±32, n=6); and 32% (±25, n=6), respectively. Work is on-going to further optimize the protocol, to reduce variability, and to functionally characterize the cardiomyocytes generated from iPS cells in this new culture system. The cardiomyocyte differentiation culture system described here is easy to use as it simply involves sequential media changes with cardiomyocyte-inducing factors in the STEMdiff™ Cardiomyocyte Media and Supplements, while maintaining the cells in their original feeder-free monolayer culture with no need for cell detachment, EB formation, or re-plating steps. In conclusion, these results demonstrate that the STEMdiff™ Cardiomyocyte Kit can be used to efficiently form beating cardiomyocytes from human iPS cells in a defined, feeder-free, monolayer culture system.

W-2334

PROCESS DEVELOPMENT FOR SCALABLE MANUFACTURING OF HUMAN ES CELL-DERIVED CARDIOMYOCYTES

Vincent C. Chen[1], Jingjing Ye[1], Hua Giau[1], Ziguang Lin[1], Danlin Chen[1], Aparna Roy[1], Patricia Huang[1], Wei Dang[1], Wenyi Chen[2], Joseph Wu[2], David Hsu[1], Larry Couture[1]

[1]Center for Applied Technology Development, Beckman Research Institute of City of Hope, Duarte, CA, United States, [2]Stanford University School of Medicine, Stanford, CA, United States

Myocardial infarction and heart failure are leading causes of death worldwide. Because the heart has very limited regenerative capacity, endogenous cell regeneration is not sufficient to repair heart damage. The concept of cell replacement has become an appealing therapy to treat such cardiac diseases. Human embryonic stem cells (hESC) are an attractive source for the cell replacement therapy since they can be greatly expanded in culture and differentiated into specific cell lineages under proper inductions. To provide sufficient hESC-derived cardiomyocytes for pre-clinical and clinical studies, a robust scalable differentiation system for cardiomyocyte production is essential. Previously we have established a scalable hESC suspension culture system with defined culture conditions. Using hESCs generated from the suspension culture system, we have developed a scalable differentiation process in adherent format, with which hESCs cultured on Matrigel are induced for cardiac differentiation by sequential treatment of Activin A and BMP4. This process has been applied to large-scale manufacturing of hESC-derived cardiomyocytes with 50-80% purity. For potential clinical application, we also develop a differentiation process with small molecule induction on cells grown on a defined matrix. Under this defined system, hESCs can be differentiated to cardiomyocytes with over 80% purity in a large scale vessel. We further use the small molecule induction approach to directly differentiate the hESC suspension culture in spinner flasks without matrix addition. With this scalable suspension culture system, we are able to obtain over 80% cardiomyocytes after differentiation. The suspension culture system from expansion of undifferentiated hESCs to cardiomyocyte differentiation provides a bioreactor prototype for automation of cell manufacturing. In summary, we have developed robust processes for scalable manufacturing of hESC-derived cardiomyocytes in both adherent and suspension culture systems that will accelerate the advance of pre-clinical and clinical studies.

W-2335

CHARACTERISATION OF CARDIOMYOCYTE PROGENITOR CELLS ISOLATED USING NOVEL MONOCLONAL ANTIBODIES DURING THE DIFFERENTIATION OF HESCS TO CARDIOMYOCYTES

Hau Wan LEUNG[1], Jayanthi PADMANABHAN[1], Marie-Josè GOUMANS[2], Andre CHOO[1]

*[1]Stem Cells, Bioprocessing Technology Institute, A*STAR, Singapore, Singapore, [2]Dept of Molecular Cell Biology, Leiden University Medical Center, Leiden, Netherlands*

The use of stem cell-derived cardiomyocytes in regenerative medicine is one of the envisioned goals of stem cell therapy. The current bottlenecks faced when deriving cardiomyocytes from pluripotent stem cells, such as human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), would be low differentiation efficiencies for certain cell lines, and heterogeneity of the cell population obtained. We propose an alternative method to circumvent these problems by enriching for cardiac-committed cardiomyocyte progenitor cells (CMPCs) partway during hESC differentiation to cardiomyocytes using monoclonal antibodies (mAbs) specific to CMPCs. The aims of this study are: firstly, to generate novel mAbs specific to CMPCs using a surrogate CMPC line isolated from human heart tissue; and secondly, to characterise CMPCs isolated using these mAbs, both from hESC differentiation to cardiomyocytes, and also from human heart tissue.
We generated a panel of mouse mAbs against raised against primary CMPCs isolated using Sca-1 from human heart tissue (A. M. Smits et al (2009), Nature Protocols, 4(2), 232-243). From the panel of antibodies generated, 11 candidate antibodies were shortlisted based on their specificity towards CMPCs, but not human fibroblast or hESC by flow cytometry and immunocytochemistry. The binding profile of these 6 candidate mAbs were tracked during hESC differentiation to cardiomyocytes at various time points. We observed a temporal expression pattern for 4

of the mAbs, which showed a gradual increase of mAb binding during the initial stages of differentiation, peaking before or at the onset of beating areas, and subsequently decreased as the differentiation proceeded.
We have used one of these mAbs, mAb C19, to enrich for CMPCs during hESC to cardiac differentiation. These cells can be expanded and maintained for at least 10 passages, and under CMPC maintenance conditions, cardiac markers in the captured population are higher than the corresponding non-binding population. With the right differentiation cues, mAb C19-sorted cells can continue to differentiate into cardiomyocytes. To understand the gene expression profile associated with these mAb C19-sorted cells, microarray studies have also been performed on CMPCs sorted from both hESC differentiation and human fetal heart tissue. From this study, we have found that these CMPCs also have higher expression of genes related to endothelial and tube development. This result has been verified by cell angiogenesis assays.
In conclusion, we have generated a panel of mAbs that are specific against CMPCs. As a pilot study, mAb C19 have been used to enrich for CMPCs during hESC differentiation. These cells retain similar proliferation and differentiation capabilities compared with Sca-1 isolated CMPCs. In addition, these cells also show a propensity to differentiate to the endothelial lineage. This supports the use of our novel mAbs in the isolation and characterisation of CMPC populations with multi-lineage capacity during hESC differentiation.

W-2336

SYSTEMATIC DISCOVERY OF TRANSCRIPTION FACTORS FOR CELL DIFFERENTIATION

Kohei Yamamizu[1], Alexei A. Sharov[1], Yulan Piao[1], Veronika Zsiros[2], Hong Yu[1], Kazu Nakazawa[2], David Schlessinger[1], Minoru SH Ko[1]

[1]National Institutes of Health, Baltimore, MD, United States, [2]National Institutes of Health, Bethesda, MD, United States

A network of transcription factors (TFs) determines cell identity, but identity can be altered by overexpressing a combination of TFs. In principle, this opens the possibility of achieving one of the goals of regenerative medicine - generating the desired differentiated cells from pluripotent stem cells, such as embryonic stem (ES) cells and induced pluripotent stem (iPS) cells. However, choosing and verifying combinations of TFs for specific cell differentiation has been daunting due to a large number of possible combinations of ~2,000 TFs. In this study, we show an unbiased approach to systematically identify TFs that can direct efficient and rapid cell differentiation. Previously, we have generated the global gene expression profiles obtained by overexpressing single TFs using the NIA mouse ES cell bank, which consists of 137 mouse ES cell lines. We start with the correlation matrix between the global gene expression responses to the induction of single TFs and the global gene expression profiles of a variety of tissues and organs. Based on the correlation matrix, we select TFs as examples and show that their overexpression differentiates ES cells into cells of specific organs, as predicted: Myod1 for skeletal muscles, Sfpi1 or Elf1 for blood cells, Hnf4a or Foxa1 for hepatocytes, and Ascl1 or Foxg1 for neurons. Furthermore, we show that transfection of synethetic mRNAs of Myod1, Sfpi1, Hnf4a, or Ascl1 generates specific target cells. These results demonstrate both the wide-ranging utility of this approach to identify potent TFs for cell differentiation, and also the unanticipated capacity of single TFs to directly guide differentiation to specific lineage fates.

W-2337

GENOMIC INSTABILITY DURING EARLY DIFFERENTIATION OF EMBRYONIC STEM CELLS

CLARA I. ESTEBAN[1], HAROLD H. MORENO-ORTIZ[2], CAROLINA LUCENA[3], DWAYNE WISE[1], NANCY REICHERT[1]

[1]BIOLOGICAL SCIENCES DEPARTMENT, MISSISSIPPI STATE UNIVERSITY, STARKVILLE, MS, United States, [A]REPRODUCTIVE GENETICS, [B]STEM CELLS UNIT, [2]FERTILITY AND STERILITY COLOMBIAN CENTER-CECOLFES, BOGOTA, Colombia

Understanding how genomic instability could be involved in the regulation and establishment of cell lineage commitment during embryonic stem (ES) cell differentiation into an embryoid body (EB) would provide crucial know-

ledge of stem cell biology. Therefore, defining the signaling pathway that controls early cell fate decisions is an important focus of research. Here, we determine the degree of instability in single tandem repeat markers located near embryonic developmental genes responsible for pluripotency, differentiation, and imprinting of the ES cells. We determined that the mean values of instability frequencies in EB from H1 and H7 ES cell lines showed significant differences across time between ES cell lines. Markers that became unstable during spontaneous differentiation showed higher instability frequencies associated with pluripotency (D1S551), differentiation (D16S3034, D16S3090, D14S588, D11S4090, D3S1583, D1S468, DXS1208, D4S2623, and D18S63), and imprinting (IGF2PROM, GRB10PROM, HISTHB2, D6S2252, D2S144, D3S1611, D7S488, and D10S529). Genomic instability influences the loss of pluripotency and the gain of cell lineage specialization. Interestingly, the differentiation potential of EBs from the two stem cell lines varied. EBs from H1 were prone to neuroectoderm differentiation in comparison to EBs from H7, which showed functional differentiation into mesoderm in the form of contractile cardiac muscle. We suggest that genomic instability in repetitive regions could be a signal for cell fate decision during differentiation among ES cell lines. Our results indicate correlation of instability in specific markers located near developmental genes and epigenetic modulators in EB that underwent spontaneous differentiation in vitro. The significance of elucidating possible molecular mechanisms of genomic instability and validation of novel biomarkers could potentially lead to use of ES cell derivatives are safe source for cell tissue replacement in clinical applications.

W-2338

ENGINEERED ADRENOMEDULLIN1 BASED NOVEL CELL-RECOGNIZABLE BIOMATERIAL IN REGENERATIVE MEDICINE

Kakon Nag[1], Kenji Kuwasako[2], Toshihiro Akaike[1]

[1]Department of Biomolecular Engineering, Tokyo Institute of Technology, Yokohama, Japan, [2]Frontier Science Research Center, University of Miyazaki, Miyazaki, Japan

Cell recognizable-biomaterials,
like cadherins, has been successfully applied in the
field of regenerative medicine
for efficient maintenance, conversion, and purification of targeted cells like
hepatocytes, neuronal cells, cardiac cells etc. from embryonic stem cells (ESC)
and induced pluripotent stem cells (iPSC). Despite the recent advancement of biomaterials
in regenerative medicine, still there are substantial needs for
novel biomaterials or engineered biomolecules to further potentiate this field.
In this study we are introducing engineered-adrenomedullin1 (AM1), a peptide
hormone-based novel biomaterial, in stem cell biology. AM1 is
a 52-residue peptide of calcitonin gene-related
peptide (CGRP) superfamily with multifaceted roles in physiology and development. It
signals
through heteromeric conformations of a member of G protein-coupled receptor
(GPCR), namely calcitonin receptor-like receptor (CLR) with the members of the
receptor activity modifying protein (RAMP) family, specifically RAMP2 and RAMP3.
We have now identified
that AM1 has critical roles in stem cell biology too. Particularly, spaciotemporal
AM1 supplementation increased mesendoderm proliferation in human ESC more than
200% over the commonly used recipe, and continued AM1 treatment significantly
enhanced endothelial cell population in the culture. We further revealed that
AM1-induced GPCR signalling enhances classical Notch1 - Delta4 pathway in ESC
and iPSC that consequently activates Hes1, which is responsible for the mesendoderm
population proliferation. Importantly, we found that AM1 has high affinity binding
sites on the surface of generated blood cells and endothelial cells. Based on this exciting
discovery we are currently working on engineering of AM1 to apply in stem cell

biology to enhance the productivity of target lineages, as well as on-site one-step purification of the target cells.

W-2341

GLUCOSE AFFECTS OSTEOGENIC ESC DIFFERENTIATION BY ALTERATIONS IN MICRORNA EXPRESSION

Anke Dienelt, Doreen Babin, Dorota Kaniowska, Nicole I. zur Nieden
Department of Cell Therapy, Applied Stem Cell Technologies Unit, Fraunhofer Institute for Immunology and Cell Therapy, Leipzig, Germany

With increasing average age of the world population and changes in nutritional habits, diabetes is becoming a more and more prevalent disease. Long-term metabolic changes associated with diabetes, such as high blood glucose levels, can lead to severe complications affecting bone regeneration and turnover. In addition, diabetic pregnancies go along with an increased risk for the fetus to develop skeletal malformations. However, the molecular mechanisms of glucose-induced teratogenesis have insufficiently been described.
In previous studies, we have reported that skeletal malformations in the offspring of diabetic mothers can be mimicked in vitro using embryonic stem cell (ESC) differentiations into osteoblasts. In exploring embryonic bone formation in vitro we showed that diabetic glucose [4.5 g/l (25 mM)] down-regulates mRNAs specific for mature osteoblasts, whereas collagen secretion and Col1 mRNA, associated with osteoprogenitors, were enhanced. Our experiments also revealed a decreased presence of osteoclasts in diabetic glucose. Together, these data led us to believe that the bone specific phenotypes seen in newborns of diabetic mothers may originate from insufficient maturation of osteoblast and -clast precursors.
Here we aimed to identify whether the impaired differentiation in high glucose concentrations was regulated by short non-coding microRNAs (miRNAs). Microarray studies were performed to reveal the expression of miRNAs at diverse time points during early murine ESC differentiation. We identified four miRNAs to be differentially expressed during early differentiation under high (25mM, diabetic) and low glucose (5 mM, physiological) conditions: mmu-mir-28, mmu-mir-125-5p, mmu-mir-199a-5p and mmu-mir-377. In order to test whether these specific miRNAs regulated differentiation fate, overexpression studies were then performed. Upon miRNA overexpression, osteogenic differentiation fate was evaluated by determining the amount of calcified matrix deposition and by analyzing osteogenic gene expression via quantitative PCR.
Overexpression of the four miRNAs significantly altered the calcification of osteogenically induced cultures and even those that were uninduced and left to spontaneously differentiate. Additionally, we found that an overexpression of the miRNAs lead to significant changes in the expression of mRNAs specific to the osteogenic lineage including Col1, osteocalcin and osteopontin. Moreover, mRNA levels for the calcitonin receptor, which is associated with an osteoclast phenotype, were also altered upon miRNA overexpression.
In summary, we were able to show that differentiation of mESCs in different glucose concentrations impacts early differentiation by affecting expression of specific miRNAs. In turn, overexpression of these distinct miRNAs leads to alterations in the formation of osteoclast and osteoblasts and is thus likely to impact the balance between those cell types during the process of osteogenesis.

W-2342

AN IN DEPTH TRANSCRIPTIONAL ANALYSIS OF HUMAN CORTICAL DEVELOPMENT USING PLURIPOTENT STEM CELLS

Joyce Van de Leemput[1], Nathan Boles[1], Patty Lederman[1], Boaz Levi[2], Vilas Menon[2], Refugio Martinez[2], Changkyu Lee[2], Shuyuan Yao[2], Ajamete Kaykas[2], Carol Thompson[2], Sally Temple[1], **Christopher A. Fasano**[1]
[1]Neural Stem Cell Institute, Rensselaer, NY, United States, [2]Allen Institute for Brain Science, Seattle, WA, United States

Many neurological and psychiatric disorders have pathophysiologies with a strong cerebral cortical component. A clearer understanding of normal human corticogenesis will provide insight into these diseases. Recent studies have

reported the derivation of cerebral cortical cells from human embryonic stem cells (hESC) that recapitulates salient features of cortical layer formation. To date, however, an in depth transcriptome analysis of how cortical layers are generated over time has not been performed. Using a differentiation paradigm modified from previous protocols and RNA-Seq, we report the first comprehensive temporal gene expression dataset encompassing the breadth of human cortical development. Applying biocomputational techniques and clustering analysis, genes that change significantly over the time-course were grouped based on specific temporal patterns of expression. Known layer markers arise appropriately within these defined temporal groups, with deep layer markers arising before superficial layer markers. Using gene ontology and pathway analysis, we linked categories and pathways associated with neural development to these groups. Finally, we found enrichment for several neurological diseases and disorders in specific gene clusters. This data set is the first to describe the dynamic transcriptome of human cerebral cortical development and provides the basis of a resource to aid studies into normal human brain development and into neurological disorders such as autism and Alzheimer's disease.

W-2343

THE HISTONE DEACETYLASE SIRT6 REGULATES STEM CELL DIFFERENTIATION

Jean-Pierre Etchegaray[1], jiho choi[1], Cesar Sommer[2], Yun Huang[4], xue wang[4], Dafne M. Silberman[1], Konrad Hochedlinger[5], Gustavo Mostoslavsky[3], Anjana Rao[6], Raul Mostoslavsky[1]

[1]MGH- Cancer Center, Mass General Hospital - Cancer Center - Harvard Medical School, Boston, MA, United States, [A]Section of Gastroenterology, Department of Medicine, [B]Gastroenterology, Department of Medicine, [2]Boston University School of Medicine, Boston, MA, United States, [3]La Jolla Institute for Allergy & Immunology, La Jolla Institute for Allergy & Immunology, La Jolla, CA, United States, [4]Department of Stem Cell and Regenerative Biology at Harvard University and Harvard Medical School, Howard Hughes Medical Institute at Massachusetts General Hospital Cancer Center and Center for Regenerative Medicine, Boston, MA, United States, [5]La Jolla Institute for Allergy & Immunology4, La Jolla Institute for Allergy & Immunology4, La Jolla, CA, United States

In mammals, chromatin dynamics plays a critical role in developmental programs and pluripotency state of embryonic stem (ES) cells. Acetylation of histone H3 at lysine 56 is an epigenetic mark implicated in maintaining pluripotency of ES cells, although its molecular mechanism and its role in development remain unknown. We and others found that the histone deacetylase SIRT6 specifically targets acetylated H3K9 (acH3K9) and H3K56 (acH3K56), which are epigenetic marks linked to activation of gene expression, and therefore, propose to determine its role in pluripotency and embryogenesis. Our data show that ES cells derived from SIRT6 knockout mice are defective in their ability to differentiate towards endoderm and mesoderm, while promoting neuroectoderm development. We found this phenotype to be associated with de-repression of the core pluripotent genes Oct4, Sox2 and Nanog, caused by the recruitment of SIRT6 and deacetylation of H3K56ac at their promoter regions. Consequently, SIRT6-depleted Embryoid Bodies (EB) exhibit abnormally high expression levels of these core pluripotent genes. Moreover, we found SIRT6 to be an upstream regulator in an epistatic pathway involving the dioxygenases TET1 and TET2, implicated in the conversion of 5-methyl cytosine (5mC) into 5-hydroxylmethyl cytosine (5hmC), which is a critical step required for DNA dimethylation, during early embryogenesis and reprogramming of induced pluripotent stem (iPS) cells. Genome-wide analysis in SIRT6 knockout ES cells unraveled neuroectoderm genes as targets for 5hmC, which is an epigenetic mark required for transcription activation. This is consistent to the neuroectoderm prone developmental defect of SIRT6-depleted EB. Importantly, we find the role of SIRT6 in differentiation of stem cells to be recapitulated in human ES cells. Therefore, we propose SIRT6 as a master regulator safeguarding the balance between pluripotency and differentiation during early stages of embryogenesis, and also as a molecular tool to elucidate novel genetic programs required for efficiency of iPS formation. Overall, the role of SIRT6 in balancing molecular and genetic programs during pluripotency and cellular differentiation, and its conserved mechanisms of action from mouse to human, could potentially give rise to novel approaches in regenerative medicine.

W-2346

DISCOVERY OF CONSENSUS GENE SIGNATURE AND INTERMODULAR CONNECTIVITY DEFINING THE STEMNESS OF HESCS

Jeffrey J. Kim, Omar Khalid, AmirHosien Namazi, Thanh Tu, Yong Kim
Laboratory of Stem Cell and Cancer Epigenetic Research, UCLA, Los Angeles, CA, United States

Objective: Molecular markers for stemness have been identified mostly by relative comparisons between undifferentiated cells versus differentiated cells to define the characteristics of stem cells including human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). The heterogeneity in molecular signatures of hESCs depending on various experimental conditions led us to question if there are any true consensus molecular markers that define "stemness". We hypothesize that there are a set of genes that are consistently altered in human embryonic stem cells (hESCs) regardless of differentiation conditions.
Methods: To identify these consensus stemness molecular markers, we have performed a microarray analysis of H1 and H9 hESCs and compared and validated our results to 19 publicly available microarray data. Using WGCNA we have constructed consensus modules to identify differentially regulated genes during differentiation.
Results: In addition to some of the well-known stemness markers such as *OCT4* and *NANOG*, we have identified potential new markers for stemness and differentiation. Moreover, we have validated some of the top downregulated markers (*LCK*, *KLKB1* and *SLC7A3*) and the top upregulated markers (*RHOJ*, *ZEB2* and *ADAM12*) upon differentiation, which were discovered from combined analyses of our transcriptomic profiling and bioinformatics of public data sets. Furthermore, we have for the first time designated stem cell markers to specific interactive networks in the genome, identifying possible interacting partners and showing how the markers themselves relate to each other by using Cytoscape and HIVE plots.
Conclusion: Identification of new stemness markers, interacting partners and interconnectivity analyses may contribute in explaining the mechanism of stem cell self-renewal and differentiation. These novel stemness markers and our bioinformatic analyses will also serve as useful tools in various applications for delineating stemness of different cell types.

W-2347

ADAPTATION OF STRIATAL IN VITRO DIFFERENTIATION OF HUMAN STEM CELLS TO HIGHER THROUGHPUT APPLICATIONS

Sandra Lubitz[1], Jamshid Arjomand[2], Jasmin Dreyer[1], Mareen Glausch[1], Stefan Jäger[1], Ryan Schönfeld[3], Celia Dominguez[3], Ignacio Munoz-Sanjuan[3]
[1]Evotec, Hamburg, Germany, [2]CHDI Management, Inc., Princeton, NJ, United States, [3]CHDI Management, Inc., Los Angeles, CA, United States

In vitro differentiation of human stem cells facilitates the generation of unlimited numbers of post-mitotic neurons as a tool for basic research, target identification, target validation, and drug discovery efforts in Huntington's Disease (HD). In order to meet requirements for high content analyses, neural conversion of human embryonic stem cells (hESC) was achieved under defined adherent conditions. The protocol described here had been further optimized to direct primitive neuroepithelia towards striatal lineage ($Dlx5^+/Gsx2^+$) and enable scale up for screening. Characterization of *in vitro* derived progenies is routinely being performed on the OPERA system, a fully automated confocal microplate reader for high content imaging and high throughput screening. This technology offers image-based quantification of cell fate-specific markers and analysis of phenotypic readouts of HD in mixed populations upon comprehensive time course analyses without the need to achieve essentially pure populations of neural cell types. Optimization of striatal differentiation was accomplished using a well characterized hESC line (H9) and this protocol will soon be applied to human stem cells expressing mutant Huntingtin. Overall, this stem cell based approach has been shown to fulfill the main criteria for lineage-specific differentiation, such as efficiency and robustness, thereby providing a sophisticated *in vitro* human cell-based system to explore mechanisms relevant to striatal commitment and for understanding HD pathogenesis.

W-2348

TBX3 DIRECTS CELL FATE DECISION TOWARDS MESENDODERM

Clair E. Weidgang[1], **Ronan Russell**[1], Tata R. Purushothama R.[3], Susanne J. Kühl[2], Thomas Seufferlein[1], Martin Zenke[4], Michelina Iacovino[5], Michael Kyba[5], Hans R. Schöler[6], Michael Kühl[7], Sebastian J. Arnold[8], Stefan Liebau[9], Alexander Kleger[1]

[A]Department for Internal Medicine I, [B]Institute for Biochemistry and Molecular Biology, [1]University Ulm, Ulm, Germany, [2]Center for Regenerative Medicine, Harvard Stem Cell Institute, Massachusetts General Hospital, Boston, MA, USA, NY, United States, [3]Department of Cell Biology,, Institute for Biomedical Engineering, Medical Faculty,Rheinisch-Westfälische Technische Hochschule, Aachen, Germany, [4]Department of Pediatrics, Lillehei Heart Institute, University of Minnesota, MN, United States, [5]Department of Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, Münster, Germany, Germany, [6]Institute for Biochemistry and Molecular Biology, Ulm University, Germany, [7]University Medical Renal Department, University Medical Centre, Centre for Clinical Research, Freiburg, Germany, Germany, [8]Institute for Anatomy & Cell Biology, Ulm University, Germany

To date, the mechanisms governing transition from the pluripotent state to the onset of differentiation are poorly understood. Recent reports demonstrated additional functions of pluripotency-associated factors during early lineage commitment. Interestingly, the T-box transcription factor Tbx3 has been implicated in the regulation of embryonic stem cell (ESC) self-renewal and in cardiac differentiation. In the current study, we report previously unappreciated roles of Tbx3 in early lineage commitment. We show that Tbx3 is dynamically expressed during specification of the mesendoderm lineages in differentiating ESCs in vitro and in developing mouse and Xenopus embryos in vivo. Our results demonstrate that Tbx3 exerts dual cell autonomous and non-cell autonomous effects, directly activating key lineage specification factors while also activating a Nodal/Smad2 signaling signature. Finally we establish that complex compensatory mechanisms are at play such that there is a functional redundancy between Tbx3 and the closely related family member Tbx2, presumably to protect the progression of normal development. Taken together, we define novel facets of Tbx3 actions and map Tbx3 as a central upstream regulator of the mesendoderm transcriptional program.

W-2351

QUANTIFYING HUMAN ENDODERM DEVELOPMENT IN TERATOMAS

Jennifer Hesson, Patricia Kensel-Hammes, Savannah Cook, Antonio Jimenez-Caliani, Laura Crisa, Carol Ware, Vincenzo Cirulli

ISCRM, University of Washington, Seattle, WA, United States

Teratomas have been conventionally used in the context of pluripotent stem cells to identify ability to generate cells of all three embryonic lineages. In this context, these lineages are usually not quantified, nor is the extent and appropriateness of development measured due to the complexities of these measures. Using naïve and primed human embryonic stem cells (hESC) we noted a difference in the ability to generate endoderm within teratomas and the extent and context of development differed between the two pluripotency stages. We describe three methods to measure endodermal developmental ability of these lines by histological section of teratomas and through in vitro assays of differentiation (directed to definitive endoderm vs. non-directed differentiation). Histological sections are measured by surface area labeled by specific antibodies, while in vitro assays are measure through FLOW for endodermal lineage markers. Our findings will be discussed in the context of garnering more valuable information from the teratoma assay than is currently recorded.

W-2352

E2F6 MEDIATED EPIGENETIC SILENCING OF GERMLINE GENES DURING THE TRANSITION FROM GROUND TO PRIMED STATE PLURIPOTENCY REQUIRES EZH2

Milena Leseva, Katherine Santostefano, Amy Rosenbluth, Naohiro Terada
Pathology, University of Florida, Gainesville, FL, United States

The transcriptional repressor E2f6 is known to negatively regulate a set of germline genes in somatic tissues and can function together with Dnmt3b in this context. In addition, E2f6 has been described as a subunit of polycomb repressive complexes with chromatin modifying activities. However, neither the precise molecular mechanisms, nor the exact timing of E2f6-mediated gene repression are fully understood. To elucidate the cross-talk between these repressive machineries we performed gene expression analysis, bisulfite DNA sequencing and chromatin immunoprecipitations in mouse pluripotent stem cells and their differentiating descendants. Initially, we demonstrate that deletion of E2f6 in mouse embryonic fibroblasts leads to promoter de-repression and hypomethylation. We show that E2f6-dependent germline promoters are first silenced and targeted for *de novo* methylation in epiblast stem cells (EpiSCs), the *in vitro* counterpart of the post-implantation epiblast. Interestingly, this occurs despite similar occupancy of E2f6 at the promoters of its target genes in both embryonic stem cells (ESCs) and EpiSCs. Consistent with these observations, stable overexpression of E2f6 or Dnmt3b in ESCs does not induce gene silencing or promoter methylation in the ground pluripotency state. We propose that additional factors are required for E2f6-mediated germline gene silencing during the transition from a ground-to-primed pluripotency state. Using a SET domain deletion mutant of Ezh2 we demonstrate the requirement of the H3K27 trimethylation activity for repression initiation of the *Stag3* and *Smc1β* promoters during terminal differentiation.

W-2353

DYNAMICS OF X CHROMOSOME INACTIVATION

Friedemann Loos, Joost Gribnau
Reproduction & Development, Erasmus MC, Rotterdam, Netherlands

In heterogametic species, dosage compensation evolved to account for the difference in expression of sex chromosome-linked genes. In placental mammals dosage compensation is achieved by inactivation of one X chromosome in female cells in a process called X chromosome inactivation (XCI). Central players in this process are the two antisense transcribed, non-coding RNAs Xist and Tsix. *Xist* expression is required to silence the X chromosome in *cis* while *Tsix* expression is involved in the down-regulation of *Xist* on the active X chromosome.
In order to study the early genetic regulation of *Xist* and *Tsix*, and to learn more about the dynamics during the onset of XCI, we created a series of reporter constructs by homologous recombination-mediated targeting in male and female murine embryonic stem (ES) cells. *Xist* and *Tsix* transcription units were replaced with sequences encoding for GFP and CHERRY, respectively, thus allowing the expression of the reporters under the endogenous promoters of these two non-coding genes. Three ES cell lines were obtained: i) Xist promoter-GFP knock-in, ii) Tsix promoter-CHERRY knock-in and iii) double knock-in on the same allele with Xist promoter-GFP and Tsix promoter-CHERRY. We also introduced CHERRY/GFP-Ezh2 fusion transgenes into these cell lines providing a direct and live read-out for PRC2 coating and heterochromatinization of the inactive X chromosome (Xi).
Differentiation of correctly targeted ES cells and expression of *Xist* and *Tsix* on the remaining wild-type allele are unperturbed. Moreover, expression of GFP and CHERRY in targeted clones appears to faithfully recapitulate the behaviour of Xist and Tsix located on the wild-type loci during the first few days of differentiation: upon differentiation, GFP is initially up-regulated until the Xist promoter on the mutant allele becomes repressed around day 3 of

differentiation because this *Xist* deletion allele is destined to become the active X chromosome (Xa). In the double knock-in lines, which allow independent tracking of Xist/Tsix, Xist is not completely silenced. CHERRY expression on the other hand is high in undifferentiated ES cells and is repressed gradually during differentiation. Initial results, however, suggest that down-regulation of the CHERRY Tsix reporter is compromised in the absence of *Xist*. CHERRY-Ezh2 fusion protein localizes to the Xi territory for a short time-window during differentiation as would be expected from earlier studies.
Live-imaging of these cell lines is feasible for extended periods of time and together with lineage tracing will yield insights into the dynamics and the interplay of *Xist* and *Tsix* in XCI. In addition, we are currently deriving mice from these ES cell lines which will enable us to follow the dynamics of *Xist* and *Tsix* expression and localization of PRC2 *in vivo* in developing embryos.

W-2354

MOSAIC TRIPLOID HUMAN EMBRYONIC STEM CELLS DERIVED FROM TRIPLONUCLEAR ZYGOTES

Ruttachuk Rungsiwiwut, Pranee Numchaisrika, Vichuda Ahnonkitpanit, Pramuan Virutamasen, Kamthorn Pruksananonda
Department of Obstetrics and Gynecology, Chulalongkorn University, Bangkok, Thailand

In general, either donated- or abnormal pronuclear formation embryos were used as the starting material for generation of human embryonic stem cells (hESCs). Previous reports demonstrated that hESCs derived from trisomic zygotes could undergo self-correction toward chromosomally normal hESCs, resulting in the diploid hESCs. Thus, these abnormal pronuclear zygotes can serve as an alternative source for deriving normal karyotype hESC lines for clinical applications.
The objective of our study was to isolate hESCs from tripronuclear zygotes in order to confirm whether the chromosomal correction could occur in the established hESCs. A total of 8 blastocysts, developed from tripronuclear zygotes were subjected to hESC isolation. Karyotypic analysis of established hESCs were performed in an early passage in order to check the chromosomal correction. The pluripotency of established hESCs were confirmed by immunostaining of the pluripotent markers, expression of the pluripotent genes by RT-PCR. Identification of the cell line was performed by analysis of DNA finger printing. The cells were allowed to spontaneously differentiate both *in vitro* and *in vivo*. In addition, hESCs were induced to undergo trophoblast differentiation by addition of 100 ng/ml of BMP-4 into the culture. The morphological changes, expression of trophoblastic genes as well as the secretion of human chorionic gonadotropin (hCG) were detected in differentiated cells.
The results of our study showed that, chromosomal correction of tripronuclear zygotes during derivation did not occur. We have successfully generated one triploid hESC line, Chula3.hES. This line carries three sets of each chromosome and shows a numerical aberration in an early passage, as demonstrated by showing the karyotype of 70 XXY, +12 and 69 XXY, +12, -15. This cell line was positively immunostained for SSEA-4, TRA-1-60, TRA-1-81 and Oct-4. RT-PCR results showed the expression of Oct-4, Nanog, SOX2, UTF, REX1, Lin28 and Nodal. This cell line differentiated into three embryonic germ layers including ectoderm, mesoderm and endoderm both *in vitro* and *in vivo*. Short tandem repeat (STR) analysis showed triple peaks in several loci that demonstrate the triploid characteristics of the Chula3.hES cell line.
After induction of trophoblast differentiation, nearly all of Chula3.hES cells flattened and enlarged prominent nuclei that resemble the early stage of trophoblast differentiation were observed. However, at Day 14, some hES-like cells still remained in the culture. The RT-PCR results showed that differentiated cells decreased the expression of Oct-4 and did not expressed Nanog and SOX2. The expression of the trophoblast marker genes Cdx2 and EOMES were increased in BMP-4 treated cells. In addition, the secretion of hCG into the culture media by BMP-4 treated cells was measured by ELISA. We found that hCG hormone was readily detected in the culture media after 7 days of differentiation and the level of hCG consistently increased over the duration of differentiation. The level of hCG measured in the culture media at Day 14 (339.4 ± 105 mIU/ml) was significantly higher than at Day 7 (5.56 ± 2.4 mIU/ml) and Day 0 (0.1 ± 0 mIU.ml) respectively, $P \leq 0.001$.

Although the chromosomal correction of triploid hESCs generated from tripronuclear zygote did not occur in the present study, this triploid hESC line will be a useful model for investigations of cell physiology, early development and the effect of chromosomal abnormalities on tumorigenicity.

W-2355

A HIGH-THROUGHPUT SCREEN TO IDENTIFY SMALL MOLECULES WHICH PROMOTES RETINAL PIGMENT EPITHELIUM DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS.

Julien Maruotti, Karl Wahlin, Valentin Sluch, Catherine Kim, Donald J. Zack
Johns Hopkins Univ, Baltimore, MD, United States

Age-related macular degeneration (AMD) is the most common cause of irreversible visual loss in the elderly in the Western world. It is a devastating disease of the retina that can lead to central blindness. It affects the retinal pigment epithelium (RPE), the layer of cells that surrounds and nourishes the neurosensory retina. RPE dysfunction cells can lead to photoreceptor dysfunction and cell death, and subsequent vision loss.
Currently, there are few if any available options to treat or replace diseased RPE cells. Cell-based transplantation strategies offer the promise of being able to restore RPE cells, thus potentially limiting vision loss. Therefore, establishing an abundant and quality source of donor cells is of utmost importance. Human pluripotent stem cells (hPSCs) may prove suitable for this purpose: significant advances have recently been made in inducing the differentiation of hPSCs toward an RPE-like cell fate and a Phase I clinical trial using such differentiated cells is currently ongoing, and several others are being planned. Nevertheless, the length and efficiency of RPE generation from hPSCs are still not optimal. We therefore sought to develop a high-throughput screen aimed at finding small molecules that could improve RPE differentiation in terms of efficiency and time course.
hPSC were maintained by clonal propagation. Using automated liquid handlers, they were differentiated in 384-well format. In DMSO treated plates, uniformly differentiated cultures were observed. In addition, high-throughput quantitative real-time PCR for key RPE markers indicated similar expression levels across the plate. hPSC can now be differentiated in the presence of compounds from small molecule libraries, and HTS qPCR used to identify hits upregulating key RPE markers.
In conclusion, we have developed a HTS qPCR approach to identify molecules that promote RPE differentiation from hPSC, and a screen to identify differentiation promoting small molecules is underway.

W-2356

HIRA IN REGULATION OF RUNX1

Debasree Dutta
Cancer Research Program, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India

Hemogenic endothelium (HE) encompasses the population of endothelial cells having hemogenic potential. HE through an endothelial-to-hematopoietic transition generates hematopoietic stem cells (HSCs). HSCs are the most desirable source of therapeutic cells for blood diseases. Earlier studies have shown that a unique balance of the transcription factors Hoxa3 (Homeobox protein Hoxa3) and Runx1 (Runt-related transcription factor 1) drives the differentiation towards endothelial or hematopoietic progenitors respectively. However, the molecular mechanisms that regulate the chromatin domain and thereby dictate the context specific expression of these transcription factors is poorly understood. Recently, Histone chaperone mediated endothelial-gene specific regulation has been reported. So, we hypothesized that in particular, Histone chaperone HIRA might be critical for the balancing of this endothelial to hematopoietic transition. So, in this study, we investigated the role of histone chaperone HIRA in modulating the function of RUNX1, the crucial regulator of HSCs generation from HE. For that, we analyzed the classical model of embryonic stem cell (ESC) differentiation in culture. Most of the evidences till date indicate that the events associated with the establishment of hematopoietic or endothelial lineage in embryoid bodies (EBs) generated from ESCs, replicate the incidences in early yolk sacs. During differentiation of mESCs towards EBs, both Runx1 and Hoxa3 expression is upregulated by day 3 whereas the ectodermal marker Nestin gets induced by day 5.

However, Runx1 is expressed ~20 fold higher than that of Hoxa3. To analyze the role of Hira, we differentiated HIRA null ESCs towards the formation of EBs. The expression of both Hoxa3 and Nestin were induced by day 3. However, EBs from HIRA null ESCs demonstrated an increase of ~3 fold expression of Hoxa3 as well as Nestin than that of EBs from control ESCs. This further establishes the previous findings that loss of HIRA is associated with increased availability of free histones that influence the induced differentiation of ESCs. Intriguingly, the expression of Runx1 was almost abrogated in EBs from null cells within day 3. We failed to differentiate these HIRA null ESCs towards HE. As, HIRA is associated with H3.3 variant of Histone H3, so Hira mediated incorporation of Histone H3.3 at the Runx1 locus might be essential to regulate the expression of Runx1 during differentiation. While RUNX1 has been implicated in coordinating vascular morphogenesis like endothelial tube formation and sprouting, so it is tempting to speculate that the fatal vascular defects associated with HIRA null mice might develop from the abrogation of RUNX1 expression.

W-2357

DIFFERENTIAL EXPRESSION OF 5HT RECEPTORS IN HESCS AND HESC DERIVED 5HT NEURONS

Athina Samara[1], Susanne Strom[2], Joel C. Glover[3]

[1]Physiology, University of Oslo, Oslo, Norway, [2]Norwegian Centre for Stem Cell Research, Oslo, Norway, [3]University of Oslo & Norwegian Centre for Stem Cell Research, Oslo, Norway

Serotonin (5-HT) is a major neuromodulatory neurotransmitter in nearly all animal species. In mammals, the physiological effects of serotonin range from regulation of subcortical sensory and motor systems, such as pain and the central pattern generator for locomotion, to regulation of higher cognitive functions, such as mood, awareness, consciousness and sleep. Deregulation of serotonin is implicated in a myriad of neuropathologies, including pain syndromes such as migraine, motor, sleep, eating and mood disorders.
Serotonergic neurons derived from human embryonic stem cells (hESCs) would provide a useful platform for standardized and quantitative studies of differentiation, synaptic function and pathogenic mechanisms.
The 5-HTRs comprise 6 families of GPCRs (5-HT1, 2, 4, 5, 6, and 7) and one ligand-gated ion channel (5-HT3). Among these, the 5-HT2A receptor is one of the best studied and its function is associated with cognition, mood, thermoregulation, sleep, cardiovascular function, and muscle contraction.
Currently, very few 5HTR ligands are subtype-selective and development of novel, more specific 5HT antagonists is needed. Pharmaceutical R&D has mainly been compromised by a lack of experimental platforms.
OBJECTIVE
To assess the expression of 5-HTRs in hESCs and hESC-derived 5-HT neurons.
METHODS
Cell culture and neuronal differentiation: In brief, H9 and HS360 hES cell lines were cultured on human foreskin fibroblasts in 20% KO-SR in KO-DMEM supplemented with bFGF.
Neural differentiation was induced by forming embryoid bodies (EBs). EBs were plated on a mixture of matrix proteins and a 6-week protocol was followed wherein cultures were supplemented with combinations of cAMP, SHH, FGF4, FGF8, BDNF, AA, NT3 and NT4 to attain serotonergic differentiation.
Real time RT-PCR was performed to asess the expression of 5-HTRs.
RESULTS
Transcripts of all 5-HT receptor subtypes are present in hESCs. All subtypes are upregulated from 30-fold to 30000-fold after directed differentiation to 5-HT neurons. By assessing expression in different lines derived from male and female embryos, gender differences in the expression of several subtypes were found in both hESCs and differentiated 5-HT neurons.
DISCUSSION
Serotonergic neurons derived from hESCs provide a useful platform for standardized and quantitative studies of pharmacological agents. Characterization of 5-HTR expression profiles is important for developing appropriate tools for 5-HTR subtype-specific assays.

W-2358

SEX SPECIFIC GENE EXPRESSION IN HUMAN EMBRYONIC STEM CELLS

Daniel Ronen, Vadim Gercenstein, Nissim Benvenisty
Hebrew Univ Jerusalem, Jerusalem, Israel

Males and females demonstrate a large variety of sex dimorphic traits most of which result from hormonal differences. However, differences between male and female embryos initiate during early development, before hormonal influence begins, suggesting the presence of genetically driven sexual dimorphisms. In order to specifically study the contribution of the Y chromosome at the earliest stages of development we compared the gene expression profiles of male and X-inactivated female human pluripotent stem cells. This approach allowed us to detect that the sex determining gene SRY is the only Y specific gene expressed in human male embryonic stem cells. Furthermore reprogramming of male somatic cells to induced pluripotent stem cells (iPSc) resulted in activation of SRY. SRYs participation in the pluripotent state was confirmed by the activation of SRY in iPSc derived from XX+SRY sex reversal patients and the activation of a reporter gene fused to the promoter region of SRY, transfected into female ES cells. In addition sorting of ES cells based on TRA1-60 expression confirmed TRA1-60 positive male cells express SRY. In order to understand the consequence of SRY expression in male pluripotent cells we compared the gene expression profile of multiple male and female samples detecting more than two hundred differential autosomal genes. Further analysis of these genes revealed more than 80 genes containing a putative SRY binding site in their promoter. Functional annotation analysis further discovered these genes are involved in developmental regulation and steroid metabolism pathways. We confirmed the differential expression of the steroid metabolism genes ABCA1 and HSD17B12 by qPCR. As estrogen exposure results in increased proliferation of ES and other cells we tock advantage of the ability of HSD17B12 to convert the inactive estrogen precursor estron (E1) to the biologically active estradiol (estrogen, E2) in order to elucidate the functional role of the differential gene expression. Exposing ES cells to estradiol (estrogen, E2) resulted in increased proliferation of both male and female cells. While exposing ES cells to estron (E1) resulted in increased proliferation of female cells only in accordance with the increased expression of HSD17B12. Cell cycle analysis revealed an increased fraction of female cells in G2/M following E1 exposure, while apoptosis analysis showed no difference between males and females. Stable expression of SRY in female ES cells resulted in converting both the estrogen involved gene expression and the response to E1 similar to male cells. In addition, we confirmed the differential expression of ID2 and CTCFL genes which are involved in regulation of muscle, lymphoid, neuronal and spermatocye differentiation all of which show dimorphic phenotypes between the sexes in later developmental stages.
Based on these results we propose that the presence of the Y chromosome and specifically SRY may drive sex specific differences in growth and differentiation properties of pluripotent stem cells.

W-2361

DIRECTING SKELETAL MUSCLE PROGENITOR CELL FATE FROM HUMAN PLURIPOTENT STEM CELLS

Richard Swan[1], Thomas Dial[2], Zheng Luo[3], Shuang Hou[3], Jinliang Peng[3], Sean Sherman[2], Rebecca Andersen[2], Cindy Malone[1], Hsian-Rong Tseng[3], April Pyle[2]
[1]Biology, California State University Northridge, Northridge, CA, United States, [A]Department of Microbiology, Immunology, & Molecular Genetics, [B]Department of Molecular and Medical Pharmacology, [2]University of California Los Angeles, Los Angeles, CA, United States

Human pluripotent stem cells (hPSCs) offer enormous potential for studying human development, for pre-clinical screening and regenerative therapies. Muscle wasting and weakness are common to many disease states and conditions including aging, cachexia and muscular dystrophies. The focus of this study is to determine how to direct skeletal muscle differentiation from hPSCs for use in muscle disorders. The research aims of this project are to determine the best approach for directing skeletal muscle fate from hPSCs without the need for genetic manipulation. We will carry out these studies using two approaches. In the first approach, we evaluate the role of somatic mesoderm patterning signals responsible for specifying myogenesis in the developing embryo towards directing muscle

cells from hPSCs. Second we will determine the myogenic potential of skeletal muscle progenitor cells (SMPCs) using a novel-reprogramming platform.
In the first approach, we have evaluated the timing and growth factor cues required for directing SMPC specification. The SMPCs were analyzed by qPCR for known SMPC markers including PAX3, PAX7 and PAX6 as a neuronal marker up to 30 days. In this manner we have seen increased SMPC but decreased expression of neuronal markers under conditions favoring SMPC specification.
In the second approach, we will determine if overxpression using a novel non-integrating/non-viral gene delivery platform can directly reprogram hPSCs towards muscle fate. The novel reprogramming system leverages the power of a self-assembly strategy using nanoparticles and a nanostructured substrate with built in molecular recognition tags that allow for local enrichment, highly efficient and on demand packaging and delivery of numerous biomolecules to cells. These experiments will provide a novel approach for directing SMPCs and will determine the myogenic differences between directly reprogrammed somatic cells or differentiated hPSCs. Thus far we have shown that we can overexpress the SMPC regulatory Pax7 and Pax3 transcription factors within the hESC cells using this platform. Ongoing studies are evaluating the in vitro and in vivo muscle potential of the directly reprogrammed cells. Understanding the cellular cues required for development of SMPCs will have great impact for use in regenerative medicine applications.

W-2362

REVEALING SPATIAL PATTERNING IN HUMAN EMBRYONIC STEM CELL DIFFERENTIATION USING MICROPATTERNED CONTROL OF COLONY ARCHITECTURE.

Aryeh Warmflash, Benoit Sorre, Eric D. Siggia, **Ali H. Brivanlou**
The Rockefeller University, New York, NY, United States

Human embryonic stem cells (hESCs) provide a window into human development but the use of these cells to study spatial patterning and axis formation has been hampered by the fact that most differentiation protocols are designed to yield homogeneous populations of cells or produce a spatially disorganized heterogeneous combination of fates. Here, we control the size and shape of hESC colonies with micropatterning technology and use this platform to analyze spatial aspects of signaling and fate acquisition both in the state of pluripotency and during differentiation. We show that cells grown in pluripotent conditions have variable levels of both signaling effectors and key pluripotency transcription factors as a result of their ability to sense the edge of the colony. Colonies grown in micropatterned culture and differentiated with BMP4 ligand produced cells from all three germ layers in spatially ordered patterns resembling those found in mammalian embryos. These patterns are highly reproducible with nearly every colony yielding identical results. We show that these patterns depend on induced activin/nodal signaling and that they form from the outside of the colony inwards. Taken together, our results establish micropatterned cell culture as a powerful system for studying cellular communication and spatial patterning during human development.

W-2363

THE C-YES TYROSINE KINASE IS A POTENT SUPPRESSOR OF ES CELL DIFFERENTIATION AND ANTAGONIZES THE ACTION OF ITS CLOSEST PHYLOGENETIC RELATIVE, C-SRC

Xiong Zhang, Malcolm A. Meyn III, Thomas E. Smithgall
Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, PA, United States

ES cells are derived from the inner cell mass of the blastocyst stage embryo and are characterized by self-renewal and pluripotency. Previous work has implicated the Src family of non-receptor protein-tyrosine kinases in the self-renewal and differentiation of mouse ES cells. At least seven members of the Src family are expressed in ES cells, with dynamic expression and activity changes during ES cell differentiation, implying distinct functions in the control of developmental fate. In this study, we used ES cells to test the hypothesis that individual members of this kinase family act in biological opposition despite their strong homology. Specifically, we examined c-Src and its

closest phylogenetic relative c-Yes for our study, because they have been independently linked to differentiation and self-renewal in previous studies. Mouse ES cell populations were generated that express low levels of wild-type, active and kinase-dead forms of c-Yes. All c-Yes transduced ES cell populations were indistinguishable from cultures of parental cells in terms of colony morphology and pluripotency marker expression. However, cultures expressing wild-type and active c-Yes had abnormal embryoid body (EB) differentiation and retained the expression of pluripotency markers. In contrast, ES cells expressing kinase dead c-Yes formed EBs indistinguishable from the controls, demonstrating that the c-Yes anti-differentiation signal requires kinase activity. To explore the interplay of c-Src and c-Yes in ES cell renewal and differentiation, we employed a chemical genetics approach previously applied to c-Src in this system. We engineered c-Src and c-Yes mutants that are resistant to A-419259, a potent inhibitor of Src-family kinases. Previous studies have shown that A-419259 treatment blocks all Src-family kinase activity in ES cells, preventing differentiation while maintaining pluripotency. Expression of inhibitor-resistant c-Src rescues the A-419259 differentiation block, resulting in primitive ectoderm-like cells. Remarkably, ES cells co-expressing the resistant c-Src and c-Yes kinases failed to differentiate in the presence of the inhibitor, demonstrating that the c-Yes signal is dominant. Our findings show that c-Yes kinase is a potent suppressor of differentiation in ES cells. We also demonstrate, for the first time, that even very closely related kinases such as c-Src and c-Yes have unique and opposing functions in the same cell type. These results suggest that selective inhibitors of c-Src vs. c-Yes activity may allow more precise pharmacological manipulation of ES cell fate.

W-2365

A RAPID, COLUMN-FREE IMMUNOMAGNETIC SEPARATION PROCEDURE FOR THE ISOLATION OF CXCR4-POSITIVE DEFINITIVE ENDODERM FROM HUMAN PLURIPOTENT STEM CELLS

Michael J. Riedel, Vesna Posarac, Yvonne Luu, Maureen Fairhurst, Karina McQueen, Terry E. Thomas, Allen C. Eaves, Sharon A. Louis
STEMCELL Technologies Inc., Vancouver, BC, Canada

The efficient generation of mature cell types from human pluripotent stem cells (hPSCs) may require a combination of specialized media along with stage-specific cell purification strategies to further enrich for desired target cells. The initial step in the differentiation of endoderm cells from hPSCs, including those of the pancreas, liver, lung, and gut, is the formation of definitive endoderm (DE). Molecular characterization of hPSC-derived DE reveals high levels of expression of CXCR4, a cell surface chemokine receptor. The expression of additional DE markers is highly enriched within $CXCR4^+$-purified cell populations. While CXCR4 is also detected on hPSC-derived mesoderm cells, most state-of-the-art DE differentiation protocols yield minimal mesoderm cell contamination. Thus immunomagnetic purification of DE cells based on CXCR4 expression may be a plausible strategy to enrich for these cells within differentiating cultures. With increased interest in the use of patient-specific human induced pluripotent stem cells (hiPSCs) for disease modeling, drug screening, and cell-based therapies, methods for efficient DE differentiation across many hiPSC lines will be required. To address the need in the field for improved and highly efficient cell culture and purification strategies for obtaining hPSC-derived DE, we developed a rapid immunomagnetic column-free method (EasySep™) for purification of $CXCR4^+$ cells derived from hPSCs.
Human embryonic stem cells (H9) or hiPSCs (WLS-4D1) were maintained under defined and feeder-free conditions in mTeSR™1 prior to differentiation toward DE. The results showed that starting cells samples with an average frequency of 76.8 ± 4.4% (n = 9) $CXCR4^+$ cells could be purified to 92.8 ± 2.0% $CXCR4^+$ cells with excellent cell recoveries (90.2 ± 7.1%). Using STEMdiff™ Definitive Endoderm Basal Medium during separation improved cell recovery and allowed enriched DE cells to be maintained continuously in a serum-free environment both in culture and subsequently during the immunomagnetic selection procedure. Purified DE retained the ability to be continued in culture and differentiated towards more specified endoderm cell types.
The propensity for DE differentiation in hPSC lines maintained or differentiated using alternative culture methods can vary significantly. The EasySep™ CXCR4 positive selection strategy reported here offers a rapid and simple-to-use protocol for the isolation of highly pure populations of hPSC-derived definitive endoderm.

W-2366

THE DYNAMICS OF OCT4A AND ITS PSEUDOGENES EXPRESSION DURING DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

Mojca Jez[1], Sakthikumar Ambady[2], Matija Veber[3], Tanja Dominko[2], Primoz Rozman[1]
[1]Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia, [2]Worcester Polytechnic Institute, Worcester, MA, United States, [3]Educell Ltd., Ljubljana, Slovenia

OCT4A transcription factor is one of the main regulators of pluripotency. It is expressed in embryonic stem cells (ES cells), embryonic carcinoma cells (EC cells) and primordial germ cells. In recent years, the expression of OCT4 has also been detected in various tumors, normal adult tissues (bone marrow, umbilical cord and peripheral blood), and even in certain adult cell lines. The OCT4 gene has three alternative transcripts: OCT4A, OCT4B and OCT4B1, which are translated into four different protein isoforms: OCT4A, OCT4B-265, OCT4B-190, and OCT4B-164. There are also at least six known OCT4 pseudogenes scattered through the genome (PG1 to PG6). Recent publications show that contrary to previous expectations some of these pseudogenes are transcribed and possibly even translated.
The aim of this study was to determine which pseudogenes are expressed in ES cells and how their expression changes during differentiation. We focused on PG1, PG3 and PG4 because of their high sequence identity to embryonic transcript.
ES cells (line H9) were differentiated in two-step differentiation protocol in the medium containing retinoic acid. For the first 5 days cells were cultured as embryoid bodies. After five days embryoid bodies were plated on tissue culture plates and differentiated for 62 days as an attached culture. Every few days RNA samples were isolated and tested for the expression of OCT4 pseudogenes. The distinction between embryonic OCT4A transcript and all its pseudogenes and between PG1, PG3 and PG4 was achieved by specific restriction analysis of RT-PCR product. The restriction method is based on the presence/absence of restriction sites resulting from SNP (single nucleotide polymorphism) in various transcripts - each SNP is specific for each pseudogene. PCR amplicons were also cloned and sequenced to confirm the accuracy of restriction method.
Undifferentiated embryonic stem cells and EC cells (NTERA and NCCIT) did not express any of the OCT4 pseudogenes. During differentiation, the expression of pluripotency markers OCT4A, SOX2, NANOG and CRIPTO-1 was down regulated and expression of pseudogenes was turned on and they were exclusively expressed in differentiated cells.
Our work is the first report about the expression of OCT4A pseudogenes 1, 3 and 4 during ES cells differentiation. Our results show that during differentiation of stem cells there is a developmentally regulated balance between the expression of embryonic genes and pseudogenes. The switch between one transcription pattern to the other depends on differentiation status of the cells. Since pseudogenes were expressed only in mature somatic cells we propose that the expression of pseudogenes can be used as a novel marker of cell differentiation status. Our data also indicates that it is necessary to critically evaluate existing published conclusions on the expression of OCT4A in different cell types, because pseudogene expression can lead to false positive results of molecular analysis.

W-2367

PROSTAGLANDIN D2 DP1 RECEPTOR MEDIATED TOXICITY IN ALS

Sophie de Boer, Evangelos Kiskinis, Kathryn Koszka, Kevin Eggan
Harvard University, Cambridge, MA, United States

Stem cells are currently used to model a wide variety of diseases, to reveal underlying disease mechanisms and eventually facilitate drug discovery. Here, for the first time, we are able to validate a stem cell based disease model *in vivo*.
We use a previously established stem cell based assay to model the neurodegenerative disease, amyotrophic lateral sclerosis (ALS), *in vitro*. Human embryonic stem cell derived motor neurons are co-cultured with primary mouse

glia of different genotypes. Motor neurons cultured onto mutant glia show a non-cell autonomous neurodegenerative effect. This effect, is specific to motor neurons and is mediated through secreted diffusible factor(s), which induce an inflammatory response.
Expression profiles of mutant glia display an upregulation of inflammatory markers, including the Prostaglandin D2 receptor DP1 (DP1). In the co-cultures, the DP1 antagonist, BW A868C protects motor neurons from the toxic effect of mutant glia. In addition, when the DP1 agonist, BW 245C, is added to wild type co-cultures a toxic effect, similar to the one produced by mutant glia is observed. Both the DP1 antagonist and agonist work solely on the glia cells, since a pre-treatment with these compounds on the glia alone, added and removed before the motor neurons are plated, is sufficient to induce a protective or a toxic effect respectively.
Since the DP1 receptor is expressed in a variety of cell types, we went on to identify the cell type that is responsible for these effects in the glia culture. We designed a modified co-culture system, in which microglia from different genotypes are spiked into the co-cultures. Mutant spiked in microglia have a toxic effect on the motor neurons, similar to the effect of the mutant glia. When these microglia were pre-treated with the DP1 antagonist, no toxic effect was observed. Indicating a direct interaction between microglia and the compound.
Furthermore, when glia derived from DP1-/- mice, carrying the ALS mutation, were co-cultured no motor neuron degeneration was observed. Additionally, an *in vivo* survival study of these mice showed a significant increase in lifespan compared to the DP1+/+ mice carrying the ALS mutation.
Collectively, our results propose for a model of ALS in which non-cell autonomous motor neuron toxicity is mediated through the Prostaglandin D2 DP1 receptor on microglia. More generally, our results validate a stem cell based disease model *in vivo* for the first time, underscoring the clinical relevance of these models.

W-2368

MESENCHYMAL STEM CELLS ENABLE ANGIOGENIC FUNCTION IN EMBRYONIC STEM CELL-DERIVED VASCULAR PROGENITOR CELLS

Lisa M. McGinley, Robert M. Nerem
Parker H. Petit Institute for Bioengineering & Bioscience, Georgia Institute of Technology, Atlanta, GA, United States

The ability to modulate vasculogenesis and angiogenesis could allow for the development of therapies for numerous physiological and pathological states associated with vessel formation, such as wound healing, tumor growth and ischemic disease. Such techniques can also be applied to the field of regenerative medicine where the development of vascular supply systems is necessary for the functionality of engineered tissues. In order to generate microvascular networks *in vitro*, a readily available and expandable source of endothelial cells capable of vessel formation is required. The derivation of endothelial cells (EC) from embryonic stem cells (ESC) offers a potential source of vascular cells and it has been suggested that bone marrow derived mesenchymal stem cells (MSCs) may function as pericytes and play an important role in regulating vascular morphogenesis. The present study aims to create mouse ESC-derived microvascular networks and investigate the interactions between MSCs and vascular progenitor cells in the context of angiogenesis.
For endothelial differentiation, mouse R1 ESC were seeded on ECM (either collagen type IV or fibronectin) for 2-5 days. Two differentiated cell populations were collected for experiments – mixed vascular progenitors (VPC) with no selection and FLK1 VPC isolated by FACS for FLK1/VEGFR2. Both cell populations were characterized by flow cytometry analysis for endothelial and smooth muscle cell markers. Next, co-culture experiments were performed to assess the effect of MSCs on angiogenesis-relevant behavior such as migration, proliferation and vessel-like structure formation in Matrigel™.
Migratory capacity of both mixed VPC and FLK1 VPC was significantly increased in response to MSCs. Increased cell number and viability was also observed when both VPC populations were co-cultured with MSCs, as well as greater proliferation. In Matrigel™, mixed VPCs alone, FLK1 VPCs alone or MSCs alone did not form networks. However, formation of microvascular-like structures did occur in the presence of MSCs. - both tubule length and number of tubule junctions was significantly increased in both progenitor cell populations. This effect was observed to be both time and dose dependent. Addition of MSCs to mixed VPCs increased network formation when both cell types were present in equal numbers. For FLK1 VPC, MSC addition after time zero in ratios of 1:1 and 2:1 (FLK1 VPC:MSC)

resulted in network formation. Further optimization of network stability in terms of MSC dose and time of addition is currently underway.
These results suggest that MSCs play an important role in angiogenesis and may enable VPC function, indicative of a pericyte role. Furthermore, terminal endothelial differentiation of ESCs may not be strictly necessary for *in vitro* microvascular network formation. In conclusion, these data may have potential implications for sourcing of functional vascular cells applicable to tissue engineering and cell therapeutics, warranting further study in the area.

W-2371

APOBEC3G INFLUENCES HEMATOPOIETIC LINEAGE COMMITMENT IN CELLS DERIVED FROM hESC

Yesai S. Fstkchyan[1], Christopher C. Nixon[2], Manik Harutyunyan[2], Cindy Malone[3], Zoran Galic[4], Jerome Zack[2]
[1]Biology, California State University Northridge, Northridge, CA, United States, [2]Department of Microbiology, Immunology, & Molecular Genetics, University of California, Los Angeles, Los Angeles, CA, United States, [3]Biology, California State University, Northridge, Northridge, CA, United States, [4]Division of Hematology and Oncology, David Geffen School of Medicine at UCLA, Los Angeles, CA, United States

Apolipoprotein B mRNA editing enzyme catalytic polypeptide like-3 G (APOBEC3G) is a member of a family of cytosine deaminases that includes activiation induced deaminase (AID), the enzyme responsible for somatic hypermutation in B cells. While APOBEC3G (A3G) is best known for its activity in restricting both retroviral infection and the mobility of genomic retrotransposable elements, there is mounting evidence that A3G possesses a far more involved role in the cell. A3G has been localized to P-bodies, subcellular foci of mRNA editing and microRNA activity and has been found to directly influence specific microRNA activity. The Broad Institute Differentiation Map (dMap) is a collection of microarray data that quantifies levels of gene expression in hematopoietic cells throughout their development. According to dMap, levels of A3G expression fluctuate tremendously through the process of hematopoiesis. This is inconsistent with a purely antiretroviral factor. We believe that A3G influences human hematopoietic lineage commitment.
Preliminary analysis by quantitative real time polymerase chain reaction (qRT-RTPCR) to analyze A3G expression at the RNA level indicates very low levels of A3G RNA in hESC but a significant rise when hESC differentiation is induced by embryoid bodies (EB). Furthermore, A3G levels are relatively hight in HSC. Together, these data suggest a role for A3G in the earliest stages of human hematopoiesis. While there is evidence that suggests A3G has a role in intermediate hematopoieitic lineage commitment, it is unclear how A3G might influence the development to HSC from embryonic stem cells (hESC). We therefore asked whether A3G plays a role in the early development of hESC into HSC.
We have successfully introduced A3G over-expression and anti-A3G shRNA constructs via lentiviral infection in H1 hESC without any overt toxicity. Stable transduction was confirmed by 4 weeks of puromycin selection and expression of our reporter gene, LNGFR. There is no apparent change in self-renewal or proliferation ability of hESC's when transduced. Transduced cells were differentiated using EB method cultured in differentiation media. EB's were differentiated for 14 days and harvested on day 15. Flow analysis of EB harvest reveals that cells with over-expression and knockdown of A3G are able to differentiate; both groups give rise to 37% CD45+ cells in similar numbers to that of the scramble control group, indicating cells with hematopoietic potential. About 2-7% of the CD45+ cell population also express CD34. Furthermore, whole EB harvests were platted into methycellulose and scored 14 days later. Knockdown of A3G showed a serve reduction of erythroid colony forming units when compared to the control group. Over expression of A3G inhibits colony formation. This is consistent with previous results where the same constructs were introduced to CD34+ HSC's from fetal liver. Specifically, when A3G expression is diminished by introduction of a lentiviral vector expressing shRNA against A3G in HSC's and assayed by CFU assays, lineage commitment is skewed in favor of myeloid lineages. In contrast, there is a 50% deficit in erythroid and megakaryocyte lineages. When A3G is over-expressed in a similar fashion, hematopoiesis is impaired. Even though further analysis is needed, preliminary data suggests that A3G plays a role in early hematopoietic develop and has no apparent role in hESC differentiation to hematopoietic progenitors.

W-2372

APOPTOTIC MECHANISM IN HUMAN EMBRYONIC STEM CELLS

Kiyoko Miyata[1], Carla M. Koehler[2], Michael A. Teitell[1]
[A]Department of Pathology and Laboratory Medicine, [B]Department of Chemistry and Biochemistry, [1]University of California, Los Angeles, Los Angeles, CA, United States

Human embryonic stem (hES) cells can differentiate into all three embryonic germ layers and may also be useful for supporting postnatal tissue renewal. They display an abbreviated cell cycle with a very short G1 phase. This acceleration in cell cycle time increases the importance for hES cells to engage mechanisms that protect genomic integrity to avoid proliferative defects that may cause aberrant development or cancer. Apoptosis is an available mechanism for maintaining genomic stability for an organism following DNA or other forms of cellular damage. Currently, it remains largely unknown what apoptotic mechanisms become engaged by hES cells under which cellular insults and also how these mechanisms are affected by hES cell differentiation. Here, we induced apoptosis in hES cells, and in hES cells induced to differentiate with retinoic acid (RA), by exposure to three different agents (actinomycin D, etoposide, and tunicamycin) that trigger distinct apoptotic pathways. We demonstrate that (a) hES cells respond more rapidly to all three insults than do RA-differentiated hES cells; (b) Bax activation and mitochondrial intermembrane space protein release, which occurs early during apoptosis, occurs more rapidly in hES cells than in RA-differentiated hES cells; and (c) cytosolic factor(s) are involved in hES cell hypersensitivity to these forms of apoptosis induction. Taken together, there is a mechanism(s) that primes hES cells for rapid death by apoptosis, which would prevent the propagation of unrepaired mutations or cellular alterations during the early critical stages of embryonic development.

W-2373

WNT5A MEDIATES CHONDROGENIC DIFFERENTIATION OF HUMAN ESC-DERIVED PROGENITORS INTO ARTICULAR-LIKE CHONDROCYTES

Jason D. Gibson, Rosa M. Guzzo, Hicham Drissi
Orthopaedic Surgery, University of Connecticut Health Center, Farmington, CT, United States

Cartilage defects often target young and middle-aged populations following traumatic joint injuries and if left untreated, damage from these injuries can lead to osteoarthritis. Treatment modalities vary depending on the severity of the cartilage damage, as well as other patient-specific factors. For large cartilage defects the gold-standard procedure is autologous cell transplantation. However, this procedure often results in the formation of fibrocartilage rather than articular-like cartilage, and the resulting hypertrophic chondrocytes in this fibrocartilage environment can produce proteases which induce matrix degradation. Thus, controlling the differentiation of progenitor cells into articular-like chondrocytes using a single growth factor would be ideal to devise a cell-based strategy to repair large articular cartilage damage. Furthermore, considering the limited regenerative capacity of adult stem cells, human embryonic stem cells (hESC) may provide an ideal cell source for more effective cell-based cartilage regeneration. Using genetic models we previously established a role for non-canonical Wnt5a signaling in the specification of chondrogenesis and inhibition of chondrocyte maturation toward hypertrophy during mouse limb development. Based on these findings, we hypothesized that Wnt5a can induce the progression of hESC-derived progenitors toward the chondrogenic lineage while inhibiting their terminal maturation. We have recently examined the chondrogenic potential of Wnt5a on the differentiation of hESCs, and the timing of Wnt5a signaling for controlling the progression of differentiating cartilage cells toward hypertrophy. Using a standard cell culture derivation technique, we induced hESCs to become mesenchymal stem cell (MSC)-like progenitors as characterized by their cell-surface expression profiles. We found that the expression profile of common MSC markers was similar between hESC-derived progenitors and de-differentiated human articular chondrocytes. We also established the multipotential ability of these hESC-derived MSC-like cells to differentiate into chondrogenic, adipogenic and osteogenic cell lineages. Chondrogenic induction of hESC-derived MSCs was achieved using high-density pellet cultures,

and the temporal effects of Wnt5a treatment in these cultures was compared to that of BMP-2. We found that while BMP-2 was able to induce differentiation of these cells into the chondrogenic lineage it also induced their terminal maturation to hypertrophy. In contrast, Wnt5a induced chondrogenic differentiation of these cells while inhibiting their terminal maturation. These effects are found to be enhanced in the hESC-derived MSC-like progenitors compared to that of the somatic human articular chondrocytes. Using low-density gene arrays, we confirmed the temporal progression of the multipotent cell populations towards a chondrogenic phenotypic in response to Wnt5a, and we established that Wnt5a not only maintained chondrogenesis while inhibiting chondrocyte hypertrophy in hESC-derived progenitors, but also induced markers of articular cartilage matrix. Together, the data indicates that non-canonical Wnt5a signaling is able to control the differentiation of hESC-derived chondroprogenitors into an articular chondrocyte-like phenotype in vitro. Experiments are currently underway to examine these effects in a translational model of cartilage repair.

W-2374

GENERATION OF BASAL FOREBRAIN AND MIDBRAIN ASTROCYTES FROM HUMAN PLURIPOTENT STEM CELLS

Federica Rinaldi, Lucy Crompton, Jennifer Badger, Maeve Caldwell
School of clinical science, Bristol university, Bristol, United Kingdom

Astrocytes are the most abundant cells in the brain and they provide trophic and metabolic support for neurons, as well as participating in crucial aspects of brain development and physiology. Historically considered as a passive and homogeneous cell, however more recently this specific type of glial cell can be classified into sub-categories according to their morphology, function, electrical proprieties and transcriptome. Different types of astrocytes are confined to certain brain regions, which clearly indicates a requirement for regional astrocytic support of local neurons and surrounding tissues. In this study we developed and validated a protocol for the differentiation of brain regional-specific astrocytes from human pluripotent stem cells including embryonic (hESCs) and induced pluripotent stem cells (iPSCs). These astrocytes will be useful to further elucidate mechanisms of developmental and neurodegenerative disease. Of particular interest is their role in regionally restricted neurodegenerative disorders, such as Alzheimer and Parkinson's diseases, which were previously exclusively considered as neuronal disorders. We have faithfully recapitulated in vivo neurogenesis using a three-stage protocol in non-adherent conditions, which allows the generation of neurons first and then of astrocytes. We use RNA and protein analysis to characterize the transition of hESCs and hIPSCs from multipotent to neural progenitor cells, and to more restricted astroglial progenitors. Ultimately, mimicking morphogen gradients occurring during neurogenesis in vivo, we successfully differentiate astrocytes with restricted regional identity, specifically basal forebrain and midbrain phenotypes. Our protocol has an estimable value for studying neurodegenerative disorders affecting a particular brain region as occurs in two of the most common neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.

W-2375

INDUCIBLE PLURIPOTENT STEM CELLS AS A TOOL IN ARTICULAR CARTILAGE REGENERATION

Wiktoria M. Suchorska[1], Tomasz Trzeciak[2], Jarosław Pawlicz[2], Magdalena Richter[2]
[1]*Medical Physics, Radiobiology Lab, Greater Poland Cancer Centre, Poznan, Poland,* [2]*and Clinics Orthopaedics and Traumatology, University of Medical Science, Poznan, Poland*

The main objective of the study was to understand the molecular mechanisms and signal transduction pathways involved in the differentiation process of the stem cells (embryonic human stem cells - hES, and induced pluripotent stem cells - IPS) into chondrocytes.
Specific objectives include:
1.Analysis of the molecular mechanisms involved in differentiation hES cells, and IPS cells into chondrocytes by analysis of global gene expression profiles at various stages of cell differentiation
2.Comparison of the mechanisms and signal transduction pathways involved in differentiation process of hES, and IPS cells to chondrocytes

3.Multidimensional comparative analysis in vitro of mature chondrocytes differentiated from both hES, and IPS cells
4.Demonstration the functionality of the hES, and IPS cells-derived chondrocytes in the regeneration of damaged articular cartilage in vivo in an animal experimental model
Since 2010, in collaboration with the Laboratory of Gene Therapy, headed by Dr. M. Wiznerowicz we perform the research project, which is successfully used a protocol based on this year's Nobel Prize winning Takahashi and Yamanaka's experience of introducing into the genome cells four transcription factors: Oct3/4, Klf4, Sox2 and cMyc using a viral vector. The results, which we obtained, confirm the possibility of maintaining in vitro full-featured IPS cells. In addition, we have established a line of human embryonic stem cells (hES). Up to the date it was proved that hES have the capacity to differentiate directly toward chondrocytes under defined cell culture conditions. During this process we controlled the morphology of the cells, and the level of gene expression typical for embryonic cells (OCT4, SOX2, NANOG), for mesoendoderm (MIXL1), for mesoderm (PDGFRbetha), for endoderm (SOX17, GATA4) and for mature chondrocytes (SOX9, CD44, collagen type II1). Moreover at the entire process of reprogramming and the differentiation of the cells culture the population of differentiated cells was examined using immunofluorescence, and immunohistochemistry (safranin O, collagen type II). We are going to perform a global analysis of gene expression at different stages of hES, and IPS cells differentiation. These analyzes based on RNA microarray technology will be made in collaboration with the Institute of Applied Cancer Center, MD Anderson, University of Texas. The experience gained by the project manager in the TCGA (Tumor Cancer Genome Atlas) Researches enable the validation of the most important genes involved in the molecular mechanisms controlling stem cells differentiation using RT-PCR.
In a further step we are going to study the protocol for differentiation IPS cells toward chondrocytes. Obtained chondrocytes will be used as a transplants for repairing cartilage damage in vivo according to the previously developed methodology. The model of in vitro analysis of regeneration the articular cartilage has been developed. Animal study are performed in collaboration with team of Dr Trzeciak from Department and Clinic of Orthopedics and Traumatology, Poznań University of Medical Sciences. We are going to examine the mechanism of repair of so-called full thickness cartilage damage which penetrates subchondral bone.
The results of the experiments will form the basis for the development of regenerative medicine and may contribute to the development of clinical protocols for future acquisition of chondrocytes from the patient's somatic cells.

W-2376

HUMAN PLURIPOTENT STEM CELLS AS A POTENTIAL SOURCE OF CLINICAL-SCALE NUMBERS OF FUNCTIONAL CHONDROPROGENITORS FOR THE REPAIR OF DAMAGED CARTILAGE

Katsutsugu Umeda, Jiangang Zhao, Brian R. Davis, Naoki Nakayama
Brown Foundation Institute of Molecular Medicine, The University of Texas Health Science Center at Houston, Houston, TX, United States

The human body is unable to repair damaged joint cartilage spontaneously, which eventually leads to osteoarthritis. Current cell-based therapies use mainly mesenchymal stromal cells (MSCs) and autologous chondrocytes, which are only available in low yield, necessitating *ex vivo* expansion. Drawbacks of expansion are the associated reduction in (re)chondrogenic activity and increased potential for fibrocartilage formation. Cartilage develops most actively during embryogenesis from three precursors: paraxial mesoderm, lateral plate mesoderm and cranial neural crest, all of which are potentially better suited to the repair of damaged cartilage than expanded adult cells. Since the early processes of *in vitro* differentiation of pluripotent embryonic stem (ES) cells mimic those of *in vivo* embryogenesis, pluripotent stem (PS) cells are the only promising source for human embryonic chondroprogenitors.
The induction of chondrogenesis from human ES cells has been demonstrated *in vitro*. However, most of the published results are based on the use of progeny generated via “uncontrolled” differentiation of ES cells, which generally display only weak chondrogenic activity. In contrast, we have reported the directed development of paraxial mesodermal progeny from human ES and induced pluripotent stem (iPS) cells in a chemically-defined medium (CDM) in the presence of defined signaling molecules that possess greater *in vitro* hyaline- cartilage forming activity than STRO1^{+}, low-passage, human bone marrow MSCs (Umeda, et al.. Sci Rep. 2012; 2:455-65).

A large portion of craniofacial bone and cartilage develop from osteochondrogenic progeny of the cranial neural crest (i.e. ectomesenchyme). In the current study, we have demonstrated that SOX9-expressing $CD271^{+}PDGFR\alpha^{+}CD73^{+}$ chondrogenic ectomesenchymal cells are selectively generated and expanded from the *SOX10*- and *FOXD3*-expressing $MIXL1^{-}CD271^{hi}PDGFR\alpha^{lo}CD73^{-}$ neural crest-like progeny of human ES cells and iPS cells in CDM supplemented with Nodal/Activin/TGFβ inhibitor and FGF. Such ectomesenchymal cells efficiently formed translucent, hyaline-like cartilage particles when "primed" with TGFβ prior to 3-dimensional pellet culture. When applied to an *in vivo* ectopic transplantation mouse model, these cartilage particles matured into mineralized cartilage, suggesting that the developed chondrocytes may be functionally similar to those in the growth plate. The ectomesenchymal cells were expandable for over 16 passages without loss of chondrogenic potential and maintained a normal karyotype for at least 10 passages. Our findings indicate that large, even clinical scale, quantities of active chondrogenic cells can be readily prepared.

In conclusion, the human PS cell-derived $CD271^{+}PDGFR\alpha^{+}CD73^{+}$ ectomesenchymal cells appear to represent an improved cell source for cell-based repair of damaged cartilage, and potentially, the basis for development of a method of cartilage-mediated bone repair.

W-2377

DIRECTED DIFFERENTIATION OF HUMAN AND MOUSE EMBRYONIC STEM CELLS TO DEFINED NEOCORTICAL SUBTYPES

Mohammad Z. Ozair[1], Gist Croft[1], Dagmar Ogrocki[2], Joanna E. Krzyspiak[1], Ali H. Brivanlou[1]

[1]Laboratory of Molecular Vertebrate Embryology, The Rockefeller University, New York, NY, United States, [2]Life Sciences and Technology, Beuth Hochschule fuer Technik Berlin, Berlin, Germany

The functional specialization of different regions of the human neocortex is dependent on the specification of projection neurons with distinct areal and laminar identities. The establishment of areal identity results in subdivision of the neocortical areas into functional fields such as sensory, visual, and motor. Laminar specification leads to the establishment of the six layers of the neocortex, a feature specific to mammalian brains. While we have a basic understanding of these processes in rodent development, whether these principles are conserved in human development remains unknown. Human embryonic stem cells (hESCs) provide a window into early human development, and have been used as a system to model early corticogenesis. Starting with Activin/Nodal and BMP inhibition, we have established a system for efficient differentiation of hESCs into all the major cortical laminar fates under defined conditions. Additionally, our results suggest that hESC derived neuroepithelium can form the three major progenitor classes found in vivo: radial glial cells, basal progenitors, and outer radial glia in a cell intrinsic manner in the absence of non-neural cells. Using live imaging and genetic tools, we observe that hESC derived neural progenitor cells (hNPCs) display many properties of radial glial cells observed in human fetal slice cultures. Moreover, hNPCs can be patterned to generate different areal identities within the neocortex using developmentally regulated morphogens. Lastly, we have also established an identical system of cortical differentiation in mouse ESCs which now permits highly efficient generation of upper cortical layers, unlike previous protocols. This platform now allows us to compare the similarities and differences in the intrinsic differentiation potential of mouse and human NPCs at the single cell level. Our experiments will allow us to gain a foothold on the molecular and cellular basis of increased complexity of the human neocortex. The ability to generate functional projection neurons with defined areal and laminar identity is a prerequisite for targeted cell therapies. By understanding the molecular underpinnings of the generation of these identities, ultimately our work will also provide a springboard for future region-specific studies on diseases involving the neocortex such as autism, schizophrenia, and Alzheimer's.

W-2378

EFFECTS OF 50 HZ MAGNETIC FIELD ON THE PROLIFERATION AND NEURONAL DIFFERENTIATION OF MOUSE EMBRYONIC NEURAL STEM CELLS

Yanwen Zhang

Third Military Medical University, Chongqing, China

Recently, several studies indicated that continuous exposure to extremely low-frequency electromagnetic fields (ELF-EMF) may affect the biologic behavior of neural stem cells (NSCs).
The aim of the present study was to investigate the possible effect of intermittent ELF-EMF exposure on NSCs. Primary cultured embryonic neural stem cells (NSCs) were exposed to a 50 Hz magnetic field (MF) at 0.5 mT, 1 mT and 2 mT for 24 h, 48 h and 72 h respectively, with intermittent cycle of 5 min on/10 min off. Cell proliferation was measureded by CCK-8 assay and Edu incorporation. Cell cycle was analyzed using a flow cytometer. The neuronal differentiation of NSCs was detected by the percentage of Tuj1 positive cells. No significant change of cell proliferation (0.5 mT, 1 mT, 2 mT for 72 h and 2 mT for 24 h, 48 h, 72 h), cell cycle(2 mT for 72 h) and neuronal differentiation(2 mT for 72 h) was observed between 50 Hz-MF-exposured and sham-exposured NSCs. Our results suggest that the present 50 Hz-MF exposure did not cause marked toxicity to NSCs.

W-2381

UAF1 REGULATES DIFFERENTIATION OF EMBRYONIC STEM CELLS.

Eunmi Park, Jungmin Kim, Kalindi Parmar, David Weinstock, Julie Sahalie, George Q. Daley, Alan D. D'Andrea
Radiation oncology, Dana-Farber Cancer Institute, boston, MA, United States

Fanconi anemia (FA) is an autosomal recessive disease characterized by bone marrow failure, congenital abnormalities, heightened cancer susceptibility and cellular hypersensitivity to DNA cross-linking agents. There are 15 FA proteins, and they co-operate in a common DNA repair pathway to promote cellular resistance to DNA cross-linking agents. Disruption of this FA pathway leads to a DNA repair disorder and to a hematopoietic stem cell defect. A central event in the FA pathway is the monoubiquitination of the FANCD2 protein. Our laboratory has identified a specific ubiquitin specific protease complex, USP1/UAF1, which controls the deubiquitination of FANCD2-Ub. UAF1 (USP1 associated factor-1) binds and stimulates the USP1 enzyme activity. In order to understand the role of USP1 in pathogenesis of FA, we have previously characterized Usp1 mutant mice. Usp1 -/- mice had elevated perinatal lethality, male infertility, cellular crosslinker hypersensitivity, and an FA phenotype. To further investigate the function of USP1/UAF1 complex, we next disrupted murine Uaf1 gene. Unexpectedly, Uaf1-/- mice exhibited early embryonic lethality. We therefore generated Uaf1 -/- mESCs (mouse embryonic stem cells). As expected, Uaf1 -/- mESCs had increased Fancd2 monoubiquitination and were hypersensitive to DNA cross-linking agent. Moreover, Uaf1 -/- mESC colonies were significantly smaller than the wild-type mESC colonies. To investigate a role of Uaf1 in regulating ES cell differentiation, we differentiated wild-type and Uaf1 -/- mESCs in vitro by leukemia inhibitory factor (LIF) withdrawal and determined mRNA expression of differentiation markers. Interestingly, at day 6 and 8 of differentiation, Uaf1 -/- mESCs displayed increased expression of genes regulating the mesoderm lineage, namely, Bmp4, Hoxa1, and Brachyury (T). In addition, at day 2 of differentiation, Uaf1-/- mESCs had increased expression of Fgf5, a primitive ectoderm marker. Since vertebrate mesoderm gives rise to a diverse variety of tissues, including the muscle, vasculature, and blood,, we are currently testing the role of Uaf1 in hematopoietic cell differentiation. Collectively, our results suggest that besides regulating a DNA repair pathway, Uaf1 and perhaps Usp1/Uaf1 complex proteins, also regulate embryonic development and differentiation.

W-2382

THE LOCALIZED FAS ON CELL SURFACE INDUCES THE DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS

Kazumi Hirano[1], Chikara Sato[2], Shoko Nishihara[1]
[1]Soka University, Hachioji, Japan, [2]Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan

Fas signaling is a major signaling of apoptosis. Fas signaling is activated by binding of Fas ligand to Fas or simply by redistribution of Fas into lipid rafts. After clustering of Fas on lipid rafts, Fas is internalized into endosomal compartments in a clathrin-dependent manner and forms receptosome beneath the cell surface. Subsequently Fas-re-

ceptosome activates caspase-8 and caspase-3. Recently, we have reported that Fas, which localized in the intracellular Golgi compartment around the nucleus, was recruited into lipid raft by heparan sulfate 3-O sulfated structure (3-O sulfated HS) on heparan sulfate proteoglycan during differentiation of mouse embryonic stem cells (mESCs). Treatment of brefeldin A, which inhibits the retrograde transport of proteins in endoplasmic reticulum and Golgi compartment, reduced the increased expression of Fas on cell surface in cells overexpressing 3-O sulfated HS, indicating that Fas was carried from Golgi compartment to cell surface. We also found that the activation of Fas signaling via 3-O sulfated HS induced the degradation of Nanog mediated by caspase-3 and differentiated into primitive endoderm and primitive ectoderm. The localization of Fas was further analyzed by atmospheric scanning electron microscopy (ASEM). The ASEM enables us to observe the sample in wet condition, such as cultured cells. The disposable dish with a silicon nitride (SiN) film window in a bottom allows various types of cells to be cultured. The use of this system permits direct observation of subcellular structures and localization of proteins of interest in wet cells at SEM resolution. To examine localization of Fas in detail, we used goat Fab' against mouse IgG doubly conjugated with 1.4 nm Nanogold and fluorescent Alexa Fluor 594 as a secondary antibody. ASEM is able to visualize samples at 8 nm resolution. The ASEM clearly showed that Fas was localized on cell surface and formed Fas-receptosome in differentiated mESCs induced by LIF withdrawal. These findings demonstrate that the localized Fas on cell surface induced the differentiation of mESCs.

W-2383

NOVEL INTEGRATIVE NUCLEAR FGFR1 SIGNALING (INFS) COMPLEMENTS PLURIPOTENCY AND CELL CYCLE MODULES IN STEM CELL DEVELOPMENT.

Christopher Terranova[1], Shridhar Narla[1], Barbara Birkaya[1], Yu-Wei Lee[1], Ewa K. Stachowiak[1], Michael Buck[2], Abhi Parikh[3], Emmanuel Tzanakakis[1], Michal K. Stachowiak[1]
[A]Pathology and Anatomical Sciences, [B]Biochemistry, [C]Chemical and Biological Engineering, [1]SUNY at Buffalo, Buffalo, NY, United States

Ontogeny requires coordinated regulation of multi-gene programs by a plethora of epigenetic signals to execute the cell transitions between self-renewal, proliferative expansion and differentiation. Systems biology postulates computational modules which integrate environmental information to control entry into the cell cycle and promote perpetual self-renewal by the stem cells. We identified an analogous Feed-Forward-And Gate module that effects postmitotic development and neuronal differentiation by the stem cells *("Stem Cells-From Mechanisms to Technologies",M.K. Stachowiak, E. S. Tzanakakis edts.World Scientific).* In the center of this module resides a novel gene-activating mechanism "Integrative Nuclear Fibroblast Growth Factor Receptor-1 (FGFR1) Signaling" (INFS). In INFS, stimulation of diverse cell surface neurotransmitter, hormonal or growth factor receptors leads to a nuclear accumulation of FGFR1, interaction and activation of limiting CBP and transcription activation. The INFS mechanism mediates cAMP-induced neuronal differentiation of multipotent human neural progenitor cells and in vivo differentiation of brain stem cells. We recently demonstrated that the activation of INFS is a common response of the pluripotent human (h) and mouse (m) ESC to all-trans Retinoic Acid (RA). .Nuclear FGFR1 is both essential for the RA induced mESC neuronal differentiation and sufficient to induce differentiation in the absence of RA stimulation. Nuclear FGFR1 associates with RXR/RAR and Nur proteins, binds to chromatin and activates gene transcription. Blocking INFS by nuclear dominant negative FGFR1(SP-/NLS(TK-) inhibits RA activation of the RARE, NurRE and NBRE enhancers while an active nuclear FGFR1(SP-/NLS) augments the RA transduction. Gene-seq indicates the INFS control of the pluripotency, neurodevelopmental and mesodermal gene programs.This study establishes further the role of the INFS signalling module that drives stem cell development. This work was supported by NYSTEM contracts C026415 and C026714.

W-2384

INHIBITION OF ROCKS ENHANCE THE FORMATION OF HEMOGENIC MESODERM AND BLOOD FROM MOUSE EMBRYONIC STEM CELLS

Michael J. Chen, Xaio Guan, Sumon M. Datta, Gloria Lee, George Q. Daley, Leonard I. Zon, Thorsten M. Schlaeger
Hematology/Oncology, Boston Children's Hospital, Boston, MA, United States

Faithfully recapitulating the key commitment stages during embryo development can facilitate direct differentiation of target tissues in vitro. In developing embryos, hematopoietic stem and progenitor cells (HSPCs) emerge from hemogenic endothelium, which is derived from Flk1+ mesodermal precursors. Mesoderm and definitive endoderm are derived from posterior and anterior primitive streak, respectively, during gastrulation. To uncover novel regulatory mechanisms and to increase the efficiency of in vitro blood derivation, we conducted a chemical genomics screen of embryonic stem cells (ESCs) carrying Flk1 reporters. We identified the Rho-associated, coiled-coil containing protein kinase (ROCK) inhibitor Y-27632 as a strong inducer for Flk1+ cell generation. Chemical inhibition of ROCK up regulates posterior primitive streak (PS) markers Evx1, HoxB1, and Tbx6, while anterior PS markers Cer1, Gsc, and Foxa2 are down regulated. Inhibition of ROCK also up regulates hematopoietic markers Lmo2, Scl, and Runx1 and increases the number of hematopoietic progenitors. Based on RNAi knockdown experiments, ROCK2 was more potent than ROCK1. Our study thus establishes ROCKs as key modulators for hemogenic mesoderm formation from ESCs.

W-2385

MONITORING CELL PROLIFERATION, DIFFERENTIATION, AND DEATH DURING ENDODERM AND ECTODERM DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS USING MULTIPARAMETER FLOW CYTOMETRY

Nil Emre, Jason G. Vidal, Lissette Wilensky, Erika A. O'Donnell, Christian T. Carson
BD Biosciences, San Diego, CA, United States

During lineage specification in vivo, there are apoptotic and cell proliferation events that accompany the differentiation of cells towards a specific lineage. In this study, we aimed to examine and monitor these events in vitro using multiparameter flow cytometry. Specifically, we examined the differentiation of human embryonic stem cells (hESCs) to definitive endoderm as well as neuronal differentiation from hESC-derived neural stem cells. We designed multicolor flow cytometry assays that enabled us to concomitantly measure and delineate the kinetics of apoptosis, cell proliferation, and differentiation. We mon-

itored active caspase-3, cleaved PARP, and histone H2AX (pS139) to measure apoptosis and DNA damage, and we utilized BrdU and DNA content analysis to monitor proliferation. Additionally, we utilized lineage-specific surface markers and transcription factors to monitor differentiation status to correlate trends in cell fate to their differentiation status. For definitive endoderm, we verified our differentiation through monitoring the down-regulation of the pluripotency marker Nanog and the up-regulation of endodermal markers Sox17, FoxA2, and CD184. For neural differentiation, Sox1, Sox2, and Ki-67 were used to identify neural stem cells, and doublecortin was used to identify neurons. Conditions for cellular fixation and permeabilization were optimized to achieve the highest resolution of all markers of interest. The simultaneous analysis of apoptosis, cell proliferation, and differentiation using multiparameter flow cytometry is an invaluable tool to both optimize differentiation protocols as well as understand cellular processes that contribute to lineage specification.

W-2386

FUNCTIONAL GENOMICS IN HUMAN EMBRYONIC STEM CELLS USING RNAI

Kevin Andrew U. Gonzales[1], Huck Hui Ng[2]
[1]NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore, Singapore, [2]Genome Institute of Singapore, Singapore, Singapore

Embryonic stem cells (ESCs), characterized by the properties of pluripotency and self-renewal, act as a good in vitro model system for early development, where the first cell lineage decisions are made. We set to perform functional genomic screens on human ESCs under various conditions with multiple readouts to identify important contributors to different aspects of human ESC biology such as self-renewal and differentiation. We started with assessing the effects of microRNA mimics on human ESCs under pluripotency-maintaining conditions to monitor microRNA-induced differentiation. Validation of the hits will determine the robustness of our system, and will enable extension of the technique to other experimental settings. Through this multi-dimensional functional genomics approach, we can create functional circuits that govern ESC processes such as early differentiation away from pluripotency and towards specific lineages.

W-2387

ASTROCYTE BASED APPROACHES TO SPINAL CORD INJURY THERAPY

Chung-Hsuan Shih, Michelle Cooney, Mark Noble, Christoph Pröschel
Biomedical Genetics, University of Rochester, Rochester, NY, United States

Every year about 300,000 patients in the U.S. suffer traumatic injury of the brain or spinal cord. Due to the limited regenerative capability of adult central nervous system (CNS), these patients are facing the debilitating consequence of their injuries. Taking advantage of the neuroregeneration promoting properties of glial cells, we have developed a cell therapy for spinal cord injury (SCI) using a specific type of astrocyte. Previously we have shown that embryonic glial-restricted precursor (GRP) cells can give rise to two distinct types of astrocyte, GDABMP and GDACNTF, upon BMP or CNTF treatment respectively. Transplantation of either rat or human origin of GDAsBMP, but not GDAsCNTF or GRPs promotes extensive neuronal survival, axonal regeneration, and functional recovery in a rat SCI model. To translate this finding into clinical application, we proceed in a two-pronged strategy: 1) to identify the regeneration promoting factors produced by GDAsBMP for future use to enhance SCI therapies; 2) to provide a more accessible source of GDAsBMP-like astrocytes by directed differentiation from human embryonic stem cells (hESCs). Differential expression analysis between GRPs and GDAs revealed a list of candidate factors that can modulate the microenvironment of injured spinal cords to encourage regeneration. Among these candidates, we identified the novel soluble factor, SF3, selectively expressed by GDAsBMP and essential for GDABMP induced neurite extension. Perturbing SF3 expression by shRNA compromised GDABMP mediated axonal regeneration after transection injury in spinal cord. Recombinant SF3 overcame adverse effect of inhibitory substrates enriched at the site of CNS lesion and promotes neurite extension in culture neurons. Lastly we have derived human astrocytes from hESCs through an intermediate neural progenitor stage. These hESC-derived astrocytes recapitulate characteristics

of GDAsBMP with respect to neuronal survival and axonal outgrowth promoting effects. These results provide clinical applicable strategies for future SCI therapy.

W-2388

GPCR SCREENING IDENTIFIES THE THROMBIN RECEPTOR PAR1 AS A NOVEL REGULATOR OF MOUSE EMBRYONIC STEM CELL (MESC) DIFFERENTIATION INTO VASCULAR ENDOTHELIUM

Min Zhang[1], Yanmin Zhang[1], Fei Li[1], Kraig Theriault[1], Haixia Gong[1], Antonia Sassmann[2], Stefan Offermanns[2], Jalees Rehman[1], Asrar B. Malik[1]

[1]Univ of Illinois at Chicago, Chicago, IL, United States, [2]Max-Planck-Institute for Heart and Lung Research, Germany, Bad Nauheim, Germany

Little is known about mechanisms that regulate differentiation of embryonic stem cells (ESCs) to endothelial cells (ECs). Here we investigated the role of G protein signaling based on our initial evidence that suppressing the Gαi signaling pathway augments vascular differentiation. By performing screeming for ~500 G protein coupled receptors (GPCRs), we identified a thrombin receptor, protease-activated receptor 1(Par1) as a candidate. Par1 is significantly up-regulated during endothelial differentiation of mouse ESCs (mESCs). Using FACS analysis, we observed that treatment of mESCs during differentiation to ECs with siPar1 or the Par1 selective antagonist SCH79797 doubled the generation of cells co-expressing the EC markers Flk1 and VE-cadherin. Quantitative PCR showed greater increases in Flk1 and VE-cadherin mRNA expression of siPar1 or SCH79797 treated cells than controls. Conversely, a Par1 agonist peptide and the physiologic Par1 agonist thrombin both significantly inhibited the differentiation of mESCs to ECs. Further dissection of the signaling pathways demonstrated that Par1 acts via the Gαi/PI3K pathway to inhibit endothelial differentiation of mESCs. We are currently generating mouse iPSCs from Par1-deficient mice to assess their capacity for endothelial differentiation. These results represent the first comprehensive screening of GPCRs that are involved in vascular differentiation of pluripotent stem cells and have identified a specific, druggable GPCR that regulates vascular differentiation. This also has important implications of vascular regeneration and repair following acute lung injury, because pluripotent stem cell derived vascular cells can augment vascular repair. Blockade of Par1 not only increases the yield of therapeutic ESC derived endothelial cells (ESC-ECs), but may also help ensure maturation and survival of transplanted ESC-ECs in situ.

W-2391

USING MOUSE EMBRYONIC STEM CELLS TO IDENTIFY MORPHOGENS AND SIGNALING MOLECULES INVOLVED IN DEVELOPMENTAL AND FUNCTIONAL ASPECTS OF GRANULAR HIPPOCAMPAL NEURONS

Maryam Hatami[1], Steffen Albrecht[1], Sabine Conrad[2], Thomas Skutella[1]

[1]Anatomy and cell biology, Heidelberg, Germany, [2]Biology and medicine university, Tübingen, Germany

Objectives: The dentate gyrus plays a crucial role in hippocampal function as the main primary afferent excitatory pathway into the hippocampus. The development of the dentate gyrus is characterized by various phases establishing a heterogeneous stem cell pool and morphogens and molecular signaling cascade that are required for distinct neurogenic steps. Since little is known about the molecular control of dentate granule cells development, research efforts have been concentrated in deducing the cascade of events that lead to the differentiation of the neuroepithelial cells into granule neurons.
Results: To characterize the role of different morphogens in development of the granule cells we have investigated the effect of morphogens such as Wnt3a, BMP-4, Dkk3a, and FGF8b.Wnt3a and FGF8b have more effect to lead differentiation into granule neurons. Some other morphology although observed such as oligodendrocytes.
Conclusions: Our results indicate that sequential treatment with morphogens may play a key role to lead differentiation of neural progenitor cells into granule neurons.

W-2392

A HUMAN INDUCED PLURIPOTENT STEM CELL MODEL OF LIGASE IV DEFICIENCY REVEALS AN IMPORTANT ROLE FOR NHEJ-MEDIATED-DSB REPAIR DURING HUMAN EMBRYONIC HAEMATOPOIESIS

Majlinda Lako[1], Katarzyna Tilgner[1], Irina Neganova[1], Sun Yung[1], Chatchawan Singhapol[2], Gabriele Saretzki[2], Jerome Evans[1], Vera Gorbunova[4], Andrew Gennery[3], Stefan Przyborski[5], Miodrag Stojkovic[6], Lyle Armstrong[1], Penny Jeggo[7]

[A]Institute of Genetic Medicine, [B]Institute for Ageing and Health, [C]Institute of Cellular Medicine, [1]Newcastle University, Newcastle, United Kingdom, [2]Department of Biology, University of Rochester, Rochester, NY, United States, [3]6.School of Biological and Biomedical Sciences, Durham University, Newcastle, United Kingdom, [4]Human Genetics Department, University of Kragujevac, Newcastle, United Kingdom, [5]Genome Damage and Stability Centre, University of Sussex,, Brighton, United Kingdom

DNA double strand breaks (DSBs) are the most common form of DNA damage and are repaired by non-homologous-end-joining (NHEJ) or homologous recombination (HR). Several protein components function in NHEJ, and of these, DNA Ligase IV is essential for performing the final 'end-joining' step. Mutations in *DNA Ligase IV* result in LIG4 Syndrome which is characterised by growth defects, microcephaly, reduced number of blood cells, increased predisposition to leukaemia and variable degrees of immunodeficiency. We report the creation of a human induced pluripotent stem cell (iPSC) model of LIG4 deficiency which accurately replicates the DSB repair phenotype of LIG4 patients. Our results demonstrate that impairment of NHEJ-mediated-DSB repair in human iPSC results in accumulation of DSBs, enhanced apoptosis and increased genomic instability, thus providing new insights into likely mechanisms used by pluripotent stem cells to maintain their genomic integrity. While haematopoietic specification of LIG4-iPSC is not affected *per se*, the emerging haematopoietic progenitors show a high accumulation of DSBs and enhanced apoptosis, resulting in reduced numbers of mature haematopoietic cells. Together our findings provide new insights onto the role of NHEJ-mediated-DSB repair in proliferation and differentiation of progenitor cells, which likely underlies the developmental abnormalities observed in many DNA damage disorders. In addition, our findings are important for understanding how genomic instability arises in stem cells and for defining appropriate culture conditions that restrict DNA damage and result in *ex vivo* expansion of stem cells with intact genomes.

W-2393

DIFFERENTIATION OF HUMAN ES CELLS TOWARDS OCULAR LENS: LENS CELL LINEAGE FORMATION, SUPPRESSION OF ALTERNATE CELL FATES, AND LENS TISSUE ENGINEERING

Rebecca S. McGreal[1], Yang Jing[1], Eric E. Bouhassira[2], Ales Cvekl[1]

[A]Department of Opthalmology and Visual Sciences, [B]Department of Medicine, Hematology, [1]Albert Einstein College of Medicine, Bronx, NY, United States

Abnormal embryonic lens development can result from a variety of genetic mutations affecting DNA-binding transcription factors, lens structural proteins, such as crystallins, and other important proteins. Such mutations may lead to congenital cataracts, which are often associated with other eye malformations. Age-related cataract is responsible for almost half of blindness world-wide. The lack of appropriate animal models as well as various limit-

ations of human primary lens cultures currently restrict the study of human cataract, therefore, the establishment of an efficient in vitro system to direct human embryonic stem (hES) cell differentiation towards lens progenitors and mature lens cells will advance studies of lens development and mechanisms of cataractogenesis.
We have previously developed a 3-stage system to differentiate hES cells into large quantities of lens-progenitor like cells and 3-dimensional lentoid bodies over 35 days of culture. Here, we optimized these conditions using various concentrations of growth factors and drugs. We found that a lower concentration of FGF2 (20 ng/mL reduced from 100 ng/mL) used to generate lentoid bodies was optimal for expression of key markers of differentiated lens. We also found that a range of FGF2 concentrations can influence hES cell fate decisions between the neuroectoderm, ectoderm/epidermis, neural crest (NC) and the pre-placodal region (PPR) as indicated by the expression levels of specific markers for the different cell types. Lentoid body cultures can now be extended to at least 70-100 days. Various drugs were tested to facilitate lentoid growth, including rho-ROCK kinase inhibitors (fasudil), drugs known to induce cellular differentiation (5-azacytidine and decitabine), as well as drugs known to act on autophagy pathways (SP600125 and etoposide). These long-term cultures can be divided into three different stages. “Early lentoids” were developed between day 18 and day 28 in the presence of FGF2 (20 ng/mL) and Fasudil (50 µM). “Intermediate lentoids” are formed between day 29 and day 35 in the presence of FGF2 (20 ng/mL), Fasudil (50 µM), 5-azacytidine (10 µM), and Decitabine (10 µM). “Mature lentoids” with improved differentiation and morphology were formed between day 35 and 70 in the presence of FGF2 (20 ng/mL) and either SP600125 (25 µM) or etoposide (20 µM).
These studies have identified improved differentiation procedures to generate lens cells and conduct long-term cultures to generate transparent 3-dimensional lentoid bodies that refract light from hES cells, an important step towards understanding both human embryonic lens development as well as human cataract.

W-2394

LHX2 REGULATES THE NEURAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS VIA TRANSCRIPTIONAL MODULATION OF PAX6 AND CER1

Hung-Chih Kuo[1], Pei-Shan Hou[1], Hong-Nermg Ho[2], Chung-Liang Chien[2]
[1]Academia Sinica, Taipei, Taiwan, [2]National Taiwan University, Taipei, Taiwan

The LIM homeobox 2 transcription factor Lhx2 is known to control crucial aspects of neural development in various species. However, its function in human neural development is still elusive. Here, we demonstrate that LHX2 plays a critical role in human neural differentiation, using human embryonic stem cells (hESCs) as a model. In hESC-derived neural progenitors (hESC-NPs), LHX2 was found to be expressed prior to PAX6, and co-expressed with early neural markers. Conditional ectopic expression of LHX2 promoted neural differentiation, whereas disruption of LHX2 expression in hESCs significantly impaired neural differentiation. Furthermore, we demonstrated that LHX2 regulates neural differentiation at two levels: first, it promotes expression of PAX6 by binding to its active enhancers, and second, it attenuates BMP and WNT signaling by promoting expression of the BMP and WNT antagonist Cerberus 1 gene (CER1), to inhibit non-neural differentiation. These finding indicate that LHX2 regulates the transcription of downstream intrinsic and extrinsic molecules that are essential for early neural differentiation in human.

W-2395

DOMINANT EFFECT OF MOUSE MUSCULAR DYSTROPHY EMBRYONIC STEM CELLS IN WILD TYPE MICE

James Patrick Gonzalez[1], Joel Schneider[1], Jessica Button[1], Corey Chang[1], Sergii Kyrychenko[1], Victoria Kyrychenko[1], Jonathan Doering[2], Mantu Bhaumik[3], Robert Grange[2], Natalia Shirokova[1], Diego Fraidenraich[1]
[1]UMDNJ, Newark, NJ, United States, [2]Virginia Tech, Blacksburg, VA, United States, [3]UMDNJ, New Brunswick, NJ, United States

We previously created chimeric mice by injecting WT mouse embryonic stem (ES) cells and induced pluripotent stem cells into mdx (dystrophin-negative) blastocysts, which are predisposed to develop symptoms of Duchenne

muscular dystrophy (DMD). We determined that a low percentage of ES cells was sufficient to supply dystrophin to the heart and skeletal muscles, producing a significant amelioration of disease (Stillwell et al. and Beck et al., PLoS one 2009 and 2011).
Recently, we generated mosaic mice by injecting mdx ES cells into WT blastocysts (termed 'reverse' chimeras). With low levels of ES cell incorporation (10-30%), the mdx/WT chimeric heart acquired dystrophic features, specifically in terms of intracellular calcium responses to mechanical stress, well before normal onset would occur in the mdx heart. While the ES-derived mdx cardiac myocytes behaved like typical mdx cardiac myocytes, surprisingly the embryo-derived WT cardiac myocytes also behaved as mdx cardiac myocytes. In addition to the effects on the heart, we observed that at a higher degree of chimerism (30-50%), some skeletal muscles like the pectoralis and diaphragm, but not the quadriceps or soleus, showed histological features of muscular dystrophy. Affected muscles displayed both a non-uniform expression of dystrophin and compromised utrophin upregulation in fibers with negligible dystrophin. Preliminary functional studies revealed that the EDL muscles, but not the soleus muscles, are sensitive to mdx ES cell chimerism, as the EDL twitch and tetanic forces, and shortening velocities were attenuated. At similar levels of chimerism, mature adipocytes showed an upregulation of muscle and cardiac markers, and evaluation of related changes in secreted hypertrophic factors such as Wnt5a and follistatin-like proteins is underway. Together, our findings suggest that the ES-derived mdx compartment dictates the overall phenotype in the heart, certain skeletal muscles, and the fat of these 'reverse' chimeras.
As mosaicism is a common feature in DMD (dystrophin+/-) carriers and in Becker muscular dystrophy, this unsuspected dominant, muscle specific function of the mdx ES-derived cells merits further studies.

W-2396

PANCREATIC-SPECIFIC EMBRYONIC MESENCHYME ENHANCES HUMAN EMBRYONIC STEM CELL DIFFERENTIATION TOWARDS PANCREATIC LINEAGES

Michael A. Bukys, Carolina Parada, Jan Jensen
Stem Cell Biology & Regenerative Medicine, Cleveland Clinic Lerner Research Institute, Cleveland, OH, United States

Human embryonic stem (hES) cells are characterized by an unlimited growth potential coupled with the capacity to form all cell-types of the adult organism. These abilities make hES cells a promising renewable source of insulin secreting cells needed for diabetes therapy. Here we test the ability of pancreatic specific mesenchyme to drive the differentiation of hES cultures towards pancreatic fates. Pancreatic tissue from E13.5 timed litters were dissected out and pooled followed by a combination of mechanical and enzymatic digestions to generate seed cultures. Two subsequent trypsin mediated passages over the course of the next four days removed evidence of epithelium components from these primary cultures as determined through RT-PCR, genomics, and cytological approaches. Initial hES differentiation experiments established that conditioned medium derived from low-passage, pancreas-specific, mesenchymal cultures (hence forward referred to as Pan-CM) had a greatly diminished capacity to sustain pluripotency of H1 hESCs as evident through decreased Oct3/4, Nanog and Sox2 expression. In addition, pluripotent cultures sustained in the presence of Pan-CM displayed an increasingly endodermal phenotype, as evident through widespread FoxA2 expression. When compared to similar mesenchymal cultures generated from lung, intestine, stomach and liver, a selective inductive ability for pancreatic fate was observed. Following two weeks of continued exposure of Pan-CM to pluripotent cultures resulted in several discrete cyst-like outgrowths and a corresponding transcript analysis demonstrated a selective up-regulation of markers associated with pancreatic progenitor cells (Pdx1, Nkx6.1, Hnf6), ductal cells (Krt 19, Ift88) and endocrine cells (Glucagon, Somatostatin). IHC analysis of the hES-derived cyst demonstrated uniform Pdx1/Hnf6 expression within DBA+ regions with limited endocrine cell development, suggesting that the Pan-CM mediated differentiation event directed hES cultures towards a pancreatic progenitor state while limiting terminal differentiation. To help determine the critical components of the Pan-CM needed to induce this pancreatic phenotype within a forward differentiating hES culture a combined analysis of the corresponding transcriptome (microarray approach) and the secretome (LC-MS approach) of the pancreas-specific mesenchymal cultures was undertaken. Several signaling molecules were identified which were uniquely expressed within the pancreas-specific mesenchymal when compared to similar mesenchymal cultures derived from lung, in-

testine, stomach, and liver. The application of such molecules, in context of a completely defined medium partially recapitulated the Pan-CM induced differentiation event.
This current study demonstrates that primary, embryonic, pancreatic-specific mesenchymal cells are capable of directly informing organ identity of pluripotent cells. The identification of a unique combination of secreted factors expressed by such cells provides a practical approach in the further exploration of optimizing induction of pancreatic fate from pluripotency.

W-2397

HUMAN PLURIPOTENT STEM CELL DERIVED MESENCHYMOANGIOBLASTS HAVE PERICYTE AND SMOOTH MUSCLE CELL POTENTIAL

Akhilesh Kumar, Saritha S. D'Souza, Igor I. Slukvin
National Primate Research Center, University of Wisconsin, Madison, WI, United States

Human pluripotent stem cells (hPSCs), either embryonic or induced, provide access to the earliest stages of human development and offer a plentiful platform for the derivation of large number of vasculogenic cells for cellular therapy and tissue engineering. We recently identified a common precursor of mesenchymal and endothelial cells, mesenchymoangioblast (MAB), as the source of mesoderm-derived MSCs in hPSC differentiation cultures. MABs can be specifically identified using serum-free FGF2-containing semisolid clonogenic medium. In these conditions, MABs form mesenchymal colonies (MS), which give rise to MSCs with osteo-, chondro-, and adipogenic potential. Here, we show that the MS colonies are capable of differentiating into pericytes and smooth muscles cells under the influence of PDGF-BB and sphingosylphosphorylcholine (SPC), respectively. Smooth muscle cells isolated and propagated from MS colonies were found to highly express specific smooth muscle cell (SMC) markers including alpha-smooth muscle actin, calponin, SM22, Myocardin (MYOCD) and smooth muscle myosin heavy chain (MYH11) and displayed strong contractile response to a carbachol and KCL vasoconstrictors. The pericytes, on the other hand, were negative for most of the smooth muscle markers, expressed high level of RGS5, CD146, CD44, CD90, CD13, and NG2 markers, and showed low levels of contractile activity as studied by time-lapse imaging. In vitro tubulogenesis assays revealed that MS colony-derived SMCs interacted with HUVECs to form short lived longer and thicker cord-like structures in vitro. The pericytes, however, are recruited to bind to capillary-like networks on the matrigel and stabilized this network. Overall, our studies demonstrated than MABs have broader differentiation potential than previously thought, and are capable of generating smooth muscle cells and pericytes in addition to MSCs and endothelial cells. Thus, MABs have a potential to provide all essential components of the vasculature and perhaps a scalable source of the entire spectrum of cells required for therapeutic vascular tissue engineering.

W-2398

DEFINED EXTRACELLULAR MATRIX COMPONENTS ARE NECESSARY FOR DEFINITIVE ENDODERM INDUCTION

Hermes Taylor-Weiner[1], Jean E. Schwarzbauer[2], Adam J. Engler[1]
[1]Bioengineering, Univ of California San Diego, La Jolla, CA, United States, [2]Princeton University, Princeton, NJ, United States

Differentiation methods often rely exclusively on growth factors to direct mouse embryonic stem cell (ESC) fate, but the niche also contains fibrillar extracellular matrix (ECM) proteins, including fibronectin (FN) and laminins (LN), which could also direct cell fate. Soluble differentiation factors are known to increase ECM expression, yet ECM's ability to direct ESC fate is not well understood. To address the extent to which these proteins when assembled into a matrix regulate differentiation, we examined mouse ESC embryoid bodies and found that their ability to maintain pluripotency marker expression was impaired by soluble serum FN. Embryoid bodies also showed a spatiotemporal correlation between expression of FN and GATA4, a marker of definitive endoderm (DE), and an inverse correlation between FN and Nanog, a pluripotency marker. Maintenance of mouse ESC pluripotency prevented fibrillar matrix production, but induction media created lineage-specific ECM containing varying amounts of FN and LN. Mouse ESC-derived matrix was unlike conventional fibroblast-derived matrix, which did not contain LN. Naïve mouse ESCs

plated onto mouse ESC- and fibroblast-derived matrix exhibited composition-specific differentiation with low ECM producing lineages showing improved differentiation on a mouse ESC-derived ECM compared to a fibroblast-derived or gelatin matrix. Fibroblast-derived matrix, when grown with exogenously added LN, is more similar in composition to mouse ESC-derived matrix and lacks residual growth factors that mouse ESC matrix may contain. Naïve mouse ESCs in DE induction medium exhibited dose-dependent DE differentiation as a function of the amount of exogenous LN in the matrix. These data imply that fibrillar FN is necessary for loss of pluripotency and that LN within a FN matrix improves DE differentiation.

W-2401

SEQUENTIALLY TARGETING DEVELOPMENTALLY EXPRESSED RECEPTORS OF THE FETAL HUMAN LATERAL GANGLIONIC EMINENCE PERMITS THE EFFICIENT INSTRUCTION OF HUMAN MEDIUM SPINY NEURONS FROM HUMAN ESCS

Su Wang, Xiaojie Li, Devin Chandler-Militello, Steven A. Goldman
CTN and Neurology, University of Rochester Medical Center, Rochester, NY, United States

Medium spiny neurons (MSNs) of the striatum are a principal cell type lost in Huntington disease (HD), a disorder characterized by a toxic gain-of-function CAG repeat expansion in the huntingtin gene. Isolated preparations of human MSNs are needed for pharmacotherapeutic screening as well as for potential cellular therapeutics. To develop an efficient protocol for generating MSNs from pluripotential stem cells, we first isolated human MSNs from fetal forebrain tissue, using DLX5 enhancer-driven EGFP-directed fluorescence activated cell sorting to isolate striatal progenitor cells from their source in the second trimester human fetal lateral ganglionic eminence. We cultured the resultant progenitor isolates, and determined that virtually all Dlx5:EGFP+ neuronal daughters differentiated as DARPP32+ MSNs. We then used Affymetrix microarrays to define the expression profiles of these human MSNs, as well as of their parental Dlx5:EGFP+ LGE progenitors; by comparing these profiles to those of SOX2+ human fetal neural progenitor cells, we established a set of genes differentially expressed during human MSN differentiation. This analysis revealed a set of receptors selectively expressed by developing MSNs, and allowed us to predict the ligands to which MSNs were differentially exposed during ontogenesis. Among these sequentially expressed MSN-lineage receptors and their predicted ligands or modulators thereof were NTRK2/BDNF, ACVR/Activin, LPR5:FZD/DKK1, PLXNA2:3/SEMA3, THRA:B/T3, INSR/insulin, and IGF1R/IGF1. Using this information, we designed a high efficiency protocol for generating MSNs from both normal (H9/WA09 and GENEA19) and huntingtin mutant (GENEA 20) human embryonic stem cells. We found that induction of the MSN phenotype was significantly potentiated by the sequential and combinatorial application of these agents, relative to extant protocols for MSN production, such that >80% of all cells in the resultant cultures, and 82 ± 2.5% of neurons, expressed a HuD+/GAD67+/DARPP32+ antigenic phenotype characteristic of MSNs. Thus, human medium spiny neurons may now be efficiently generated from human ESCs, including both normal and HD mutant hESC lines; these ells should permit a hitherto unachievable level of molecular analysis of both normative MSN biology, and its disruption in HD.

W-2402

FUNCTIONAL ROLE OF MST1/MST2 IN EMBRYONIC STEM CELL DIFFERENTIATION

LI PENG
Chemical Pathology, The Chinese University of Hong Kong, Hong Kong, China

Abstract: Mammalian sterile 20-like kinases 1 and 2 (Mst1/Mst2) are key components of Hippo pathway which is implicated in cell proliferation, apoptosis and organ size control. Recent research suggests that Mst1/Mst2 contribute to restrain tissue specific stem cell compartment in organ. To understand the role of Mst1/Mst2 in stem cells and harness stem cells for better therapeutic application, we derived M*st1/Mst2* knockout embryonic stem cells (*Mst-/-* ES cells) to systematically esplore their lineage differentiation potential. We found that *Mst-/- ES cells* express low level of phosphorylated Yap compared to wild type ES cells, suggesting Hippo pathway is functional in ES cells. *Mst-/-* ES cells cells proliferate faster than wild type cells and show differentiation resistance after LIF with-

drawal. Interestingly, although *Mst-/-* ES cells form embryoid body (EBs) and differentiate into tissues of three germ layers, they do not form teratoma. Genome-wide RNA-seq and microarray analysis revealed that canonical Wnt signaling is significantly repressed in *Mst-/-* EBs. Besides, although *Mst-/-* ES cells can differentiate into mesoderm cells, they fail to differentiate into cardiac progenitor cells. These results would enable us to explore pharmacological alteration of Mst function to regulate cardiac progenitor cell development and prevent teratoma formation during ES cell application in future.
Keywords: Hippo pathway, *Mst1/Mst2* knockout ES cell, Pluripotency, Lineage differentiation, Wnt pathway.

W-2403

REGULATION OF EMBRYONIC STEM CELL PLURIPOTENCY BY GLYCOGEN SYNTHASE KINASE-3

Mansi Shinde, Peter S. Klein
Perelman School of Medicine at UPENN, Philadelphia, PA, United States

Embryonic Stem cells (ESCs) are characterized by their ability to self-renew indefinitely, while retaining the capacity to differentiate into all lineages. The ability to control cell fate decisions possesses great therapeutic potential for regenerative medicine, and provides a valuable research tool. Glycogen synthase kinase-3 (GSK-3) has been implicated as a key regulator of pluripotency in ESCs. Although short-term GSK-3 inhibition in mouse ESCs enhances self-renewal through Wnt activation, cells cultured with GSK-3 inhibitors alone eventually succumb to differentiation. Although GSK-3 is best-known for its role in Wnt/β-catenin signaling, it functions in several other growth factor signaling pathways that have not been studied in ESCs. We hypothesize that GSK-3 inhibition promotes differentiation through activation of mechanistic Target of Rapamycin (mTOR). Our lab has previously shown that, in mouse hematopoietic stem cells (HSCs), GSK-3 regulates both self-renewal, through Wnt/β-catenin signaling, and lineage commitment, through mTOR. mTOR is a key nutrient sensing pathway that integrates extracellular and intracellular signals to regulate many cell processes, such as protein translation and autophagy. We hypothesize that mTOR promotes lineage commitment, as biosynthetic capacity and nutritional status may play a role in pluripotency. The objective of this study is to assess whether GSK-3 regulates pluripotency through Wnt/β-catenin signaling, and inhibits lineage commitment through mTOR in ESCs. Specifically, we are testing if GSK-3 inhibition activates mTOR in ESCs and whether this increase in mTOR activity contributes to differentiation. In *Gsk-3α/Gsk-3β* double knockout (*Gsk-3* DKO) ESCs and in ESCs treated with GSK-3 inhibitor, CHIR9901, we observe markedly increased mTOR activity compared to E14 wild-type ESCs, consistent with known GSK-3 suppression of mTOR in other cell types. Furthermore, when wild-type ESCs are removed from nutrient rich medium and induced to differentiate, mTOR activity initially decreases, but is restored as differentiation progresses. In contrast, mTOR and Wnt/β-catenin activity remain high in *Gsk-3* DKO ESCs, which maintain expression of the pluripotency markers for up to 14 days after induction of differentiation. Our studies in mouse ESCs support a role for a GSK-3/mTOR signaling axis in ESC differentiation.

W-2404

NEUROGENESIS OF HUMAN CORTICOSPINAL MOTOR NEURONS

Anders Smith[1], Sandra Klein[1], Arturo Zapata[1], James Weimann[2], Binhai Zheng[1]
[1]Neurosciences, UCSD, San Diego, CA, United States, [2]Neurosurgery, Stanford University, Stanford, CA, United States

Corticospinal Motor Neurons (CSMNs) send axonal projections from the sensorimotor cortex to the spinal cord and are vital for skilled voluntary movement in humans. They are damaged or undergo degeneration in spinal cord injury and amyotrophic lateral sclerosis (ALS) respectively, and thus are a clinically important population of cortical projection neurons. Due to the poor regenerative capabilities of the human central nervous system (CNS), treatments for afflicted patients are limited and nervous system damage is often permanent, resulting in chronic paralysis and other difficulties. Despite the extensive work performed in mouse models, CSMN development in humans is poorly understood. The pluripotent nature of human embryonic stem cells (hESCs) renders them an ideal vehicle to address this major gap in our knowledge base.

Developmental studies in mice have identified Fezf2 as an important marker and fate regulator of the subcerebral projection neurons (including CSMNs). Our lab has previously generated a Fezf2:EYFP hESC reporter line via homologous recombination, placing EYFP (enhanced yellow fluorescent protein) under the control of the endogenous human Fezf2 promoter. Using a differentiation protocol based on dual SMAD inhibition, we have been able to differentiate these genetically labeled hESCs toward a CNS phenotype, and have been successful in generating a relatively high percentage of Fezf2 positive neural precursors and neurons. These were initially evaluated by immunostaining at different time points throughout the differentiation process. Fezf2 positive and negative cells were then further assessed by FACS, qRT-PCR, and time-lapse imaging. Finally, to test the potential of these cells in vivo, Fezf2 expressing cells were transplanted into the cortices of neonatal mice. Through these methods, we have begun to elucidate some of the first aspects of the molecular identity and fate choice for human CSMNs.

Totipotent/Early Embryo Cells

W-2411

ESTABLISHMENT OF EFFICIENT CONDITIONS FOR SINGLE BLASTOMERE AND WHOLE EMBRYO DEVELOPMENT USING CO-CULTURES

Jasmin Jasmin, Martha de Oliveira Guerra, Vera Maria Peters
Biology Center of Reproduction, Federal University of Juiz de Fora, Juiz de Fora, Brazil

Embryonic stem cells (ESC) are one of the most promising stem cell sources for cell therapy and regenerative medicine. Recently, ESC lines were established from single blastomeres; this method could be used to reduce ethical concerns over harm to embryos but the derivation success rate is low. To derive human or mouse ESC usually a feeder layer from animal source [mouse embryonic fibroblast (MEF)] has been used. However, to the best of our knowledge mesenchymal stem cells (MSC), which secrete several growth factors, and can be easily isolated from human or mouse tissue, have not been tested in ESC derivation. In addition, despite the advances in assisted reproduction techniques, failures in in vitro embryo development continue to occur and approaches such as co-cultivation of whole embryos with MEF or MSC has not been explored. In the present study we have used co-culture of MSC or MEF to cultivate single blastomeres and whole embryos of mice. MSC and MEF were isolated and cultured in DMEM-F12 with 10% fetal bovine serum until reached the third passage. All the embryos were obtained ~42 hours (2nd day) after mating, and the single blastomere or whole embryos were randomly split in the follow groups: CTRL - cultivated in control culture medium; MSC - co-cultivated with MSC; MSCinat - co-cultivated with MSC inactivated for proliferation; and MEFinat - co-cultivated with MEF inactivated for proliferation. The inactivation process was performed due to the high proliferation rate of these cells using mitomycin C. The development of single blastomere or embryo cultures was evaluated daily for 5 days (7th day after mating). We observed at 2 days after mating (day of embryo acquisition) the following stages of development: 87.0% at 2-cell, 6.5% at 3-cell, 3.7% at 4-cell and 2.8% at 5-8-cell. Single blastomeres co-cultivated with MSC, MSCinat or MEFinat formed ESC-like colonies after the 4th day of culture, which was not observed in CTRL condition. In addition, we noted with interest that the blastomeres co-cultivated with MSC or MSCinat had a greater development at all time points and formed a higher number of ESC-like colonies than when co-cultivated with MEFinat (57.1, 42.9 and 28.6% for MSC, MSCinat and MEFinat respectively) on the 5th day of culture. For whole embryo cultures we observed that embryos co-cultivated in MSC, MSCinat or MEFinat groups showed a greater development than in CTRL group after the 3rd day of culture. On the 5th day culture the rate of hatched blastocysts in MSC, MSCinat and MEFinat groups were 23.3, 33.7 and 58.2% higher, respectively, than in CTRL group. In summary, our preliminary data show that MSC seems to be a better feeder layer to obtain ESC-like colonies from single blastomere cultures and both MSC and MEF are able to greatly improve the early embryo development in vitro. Supported by FAPEMIG (BPD-00021-12 and REDE26/11).

W-2412

ATYPICAL PKC, REGULATED BY RHO GTPASES AND MEK/ERK, PHOSPHORYLATES EZRIN DURING EIGHT-CELL EMBRYO COMPACTION

Hongjie Liu[1], Zhaoting Wu[1], Xianle Shi[1], Wenzhi Li[2], Lin Liu[1], Jie Na[2], Hong Cheng[3], **Lingyi Chen**[1]
[1]Nankai Univ, Tianjin, China, [2]Tsinghua Univ, Beijing, China, [3]Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Phosphorylation of Ezrin T567 plays an important role in eight-cell embryo compaction. Yet, it is not clear how Ezrin phosphorylation is regulated during embryo compaction. Here, we demonstrated that inhibition of Mek/Erk or protein kinase C (PKC) signaling reduced the phosphorylation level of Ezrin T567 in eight-cell compacted embryos. Interestingly, the Rho GTPase inhibitor C3-transferase caused basolateral enrichment of atypical PKC (aPKC), as well as basolateral shift of phosphorylated Ezrin, suggesting aPKC may be a key regulator of Ezrin phosphorylation. Moreover, inhibition of PKC, but not Mek/Erk or Rho GTPases, affected the maintenance of Ezrin phosphorylation in compacted embryos. We further identified that aPKC is indeed required for Ezrin phosphorylation in eight-cell embryos. Taken together, Rho GTPases facilitate the apical distribution of aPKC and Ezrin. Subsequently, aPKC and Mek/Erk work together to promote Ezrin phosphorylation at the apical region, which in turn mediates the apical enrichment of filamentous actin, stabilizing the polarized apical region and allowing embryo compaction. Our data also suggested that aPKC might be the Ezrin kinase during eight-cell embryo compaction.

Germline Cells

W-2421

UNDERSTANDING THE CELL CYCLE IN PRIMORDIAL GERM CELL DEMETHYLATION

Joseph D. Hargan Calvopina, John Vincent, Kevin Nee, Amander Clark
MCDB, UCLA, Los Angeles, CA, United States

The germ line epigenetic ground state is established in germ line progenitors called primordial germ cells (PGCs). This epigenetic landscape is necessary for the production of gametes, and to prevent the inheritance of epialleles to future generations. One major characteristic of the germ line epigenetic ground state is genome-wide depletion of cytosine methylation from the genome. We, and others have shown that depletion of cytosine methylation in PGCs occurs in two phases, the first phase involves a global depletion of cytosine methylation that removes methylated cytosines genome wide. In humans this occurs before 6 weeks, and in mice before e9.5. This is followed by a second phase of demethylation, which occurs in a temporal and locus-specific manner. The second phase is regulated in part by the Ten eleven translocation (Tet) proteins Tet1 and Tet2 and conversion of 5-methylcontisine (5mC) to 5-hydroxymethylcytosine (5hmC) at specific loci. The major working model for Tet-dependent DNA demethylation involves replication-coupled loss of methylated cytosines from the genome. However an alternate model would predict active removal of 5hmC at specific loci independent of cell division. In order to address this directly, we have established a new organ culture model involving the growth of dissected aorta/gonad/mesonephros (AGM) tissues isolated from the mouse embryo at e10.5. During three days of organ culture, we show that PGCs divide on average three times. We also show that in the background of global hypomethylation established in phase 1, PGCs isolated from the organ culture undergo locus-specific DNA demethylation, and this occurs within three days. Using this model we have targeted the PGC cell cycle using chemical inhibitors, and are currently evaluating the effects of cell cycle inhibition on locus-specific DNA demethylation. Results from this work have important implications for understanding the control of DNA methylation in the germ line as well as during embryo development and nuclear reprogramming.

W-2422

EVIDENCE OF PRIMORDIAL GERM CELL MARKERS IN ADULT LAGOSTOMUS MAXIMUS OVARY - AN ENDEMIC MAMMAL OF SOUTH AMERICA

Nicolas A. Fraunhoffer, Analia Meilerman Abuelafia, Pablo IF Inserra, Noelia P. Leopardo, Alfredo D. Vitullo
Centro de Estudios, Biomédicos, Biotecnológicos, Ambientales y Diagnóstico – CEBBAD –, Universidad Maimonides, Buenos Aires, Argentina

BACKGROUND: A central dogma of mammalian reproductive biology is that females are born with a finite, non-renewing pool of germ cells, all of them arrested in meiosis I (oocytes) and enclosed within follicles. In 2004, studies in mice challenged this dogma. It was concluded that the adult mouse ovary retains rare female germ cells or oogonia stem cells (OSCs) that can potentially generate oocytes de novo, a situation already described in prosimian since the 1970s´. More recently, in 2012, Tilly and coworkers identified and isolated OSCs from ovaries of reproductive-age women. Whether or not OSCs remain active in the adult mammalian ovary is a matter of current debate with studies showing evidence for and against oogenesis de novo. The South American plains vizcacha, L. maximus, is a hystricognathe rodent that shows the highest ovulation rate described for a mammal. Females release 400 to 800 oocytes in each estrus cycle accompanied by a considerable number of non-ovulated oocytes remaining inside luteinizing follicles. Moreover, oogenesis doesn´t stop during gestation; an ovulatory process takes place at mid-gestation with massive formation of secondary corpora lutea, and reproductive life spans over 10 years. In such a dynamic ovary, oogenesis de novo may well account for the permanent rebuilding of tissue organization and germ cell supply.
OBJECTIVE: To search for OSCs throughout the reproductive cycle in the adult ovary of L. maximus by expression analysis of primordial germ line markers.
MATERIAL AND METHODS: This study was reviewed and authorized by the institutional Committee on the Use and Care of Experimental Animals. A total of 25 adult ovaries belonging to non-pregnant ovulating females (OF; N=5); females at early-(EG; N=5), mid-(MG; N=5) and late-gestation (LG; N=5), and post-partum females (PP; N=5) were studied. All samples were processed by immunohistochemistry, immunofluorescence, western blot (WB) and real time PCR (qPCR) for DDX4, IFITM-3, STELLA, BLIMP-1, DAZ-L and OCT-4.
RESULTS: Pluripotency marker OCT-4 was strongly detected in surface epithelium and clusters of cells inside the ovary, especially in LG and PP ovaries, and confirmed by western blot and qPCR. Germ cell markers DDX4 and DAZ-L were expressed in cytoplasm of oocytes contained in primordial follicles in all groups. Both germ cell markers were observed in clusters of oocytes in PP period. IFITM-3 and STELLA expression had a membrane and cytoplasmic localization, respectively, and were associated especially with the surface epithelium and the tunica albuginea. The WB and qPCR showed a slight increase in LG and PP ovaries. BLIMP-1 showed a nuclear distribution in some cells associated to the surface epithelium. It was confirmed by WB and qPCR.
CONCLUSION: These results support the existence of potential OSCs in the mature ovary of L. maximus by showing cells expressing pluripotency and germ cell-specific markers associated to the surface epithelium and in cell clusters inside the ovary. It is worth to note that cells expressing OSCs markers are organized in clusters especially in LG and PP females which need to reorganize the ovarian reserve for the next reproductive season.

W-2423

GERM CELL PROFILING REVEALS MOLECULAR SIGNATURES

Jia Hui Ng
Genome Institute of Singapore, Singapore, Singapore

Germ cells are unipotent cells that give rise to mature gametes. In the developing embryo, germ cells undergo genome-wide reprogramming that erases and also re-establishes DNA methylation and certain histone marks. Remarkably, germ cells retain the plasticity to acquire pluripotency *in vitro* if given the appropriate culture conditions. While it is known that early fetal germ cells express some pluripotency genes such as *Pou5f1*, the epigenome of germ cells is not well understood. Here, we performed transcriptional profiling and chromatin immunoprecipitation coupled with high-throughput sequencing (ChIP-Seq) on fetal germ cells that were purified from mouse embryonic gonads. Our data shows that the E11.5 germ cells express many pluripotency-related genes that are soon downregulated by E15.5. Comparison of the active histone mark H3K27ac also reveals *cis*-regulatory regions that are similarly and differentially marked in germ cells and pluripotent mouse embryonic stem cells (ESCs). Interestingly, germ cells share many bivalent sites (marked by both H3K4me3 and H3K27me3) with ESCs, with a greater overlap than when compared to the other cell types. We also observed classes of genomic regions that are marked by the repressive H3K27me3 histone modification in germ cells only and are enriched for retrotransposon repeats.

Further study of these germ cell transcriptional and epigenetic features may provide clues to the regulatory mechanisms that define germ cells.

W-2424

TRANSCRIPTIONAL REGULATION OF OVARIAN GERMLINE STEM CELL DIFFERENTIATION IN MAMMALS BY STEROID HORMONE SIGNALING

Chonthicha Satirapod, Ning Wang, Yvonne A. R. White, Dori C. Woods, Jonathan L. Tilly
Obstetrics and Gynecology, Massachusetts General Hospital, Boston, MA, United States

Recent studies show that adult mammalian ovaries possess germline or oogonial stem cells (OSCs) that generate oocytes *in vitro* and fertilization competent eggs *in vivo*. Genetic mouse models confirm OSC differentiation activity in adult ovaries and reveal that temporal disruption of this process causes a reversible reduction in oocyte numbers. These data indicate that, contrary to the longstanding belief mammalian females are born with a fixed pool of oocytes, maintenance of the adult ovarian reserve requires active input of new oocytes from OSCs. However, the molecular mechanisms regulating OSC activity are not known.
Steroid hormones coordinate many facets of mammalian reproduction, including the reproductive cycle in adult females. This cycle consists of an estrogen (E2)-dominant follicular phase and progesterone (P4)-dominant luteal phase. Our work and work from others show that oocyte numbers fluctuate during reproductive cycle, with lower numbers of oocytes observed during the follicular phase. Interestingly, a meiosis-initiating gene, *stimulated by retinoic acid gene 8* (*Stra8*), is also more frequently detected during this stage, suggesting a possible ongoing process of meiotic differentiation of OSCs to replenish the waning oocyte pool during each reproductive cycle. Here we show that E2 and P4 interact to regulate OSC differentiation by transcriptionally modulating expression of *Stra8*. E2 treatment of adult female mice leads to a rapid expansion of oocyte numbers accompanied by *Stra8* activation, and these effects can be blocked by P4. Mechanistic studies show that OSCs express estrogen receptor α (ERα) and progesterone receptor (PR) but not estrogen receptor β (ERβ). Importantly, ERα rapidly occupies the *Stra8* promoter on a putative estrogen response element (ERE) upon E2 treatment, supportive of ERα-mediated direct transcriptional activation of *Stra8* downstream of E2 signaling. Genetic studies confirmed a central role for ERα in mediating E2-induced OSC differentiation in that the adult oocyte pool in *ERα* knockout (*ERαKO*) mice failed to increase in number following E2 treatment; by comparison, *ERβ* knockout (*ERβKO*) mice responded to E2 like wild-type mice. In addition, while *ERαKO* mice show no developmental defects in embryonic germ cell differentiation nor in endowed oocyte numbers at birth, adult *ERαKO* mice exhibit a diminished oocyte reserve and a lower level of *Stra8* expression compared with age-matched wild-type and *ERβKO* mice. Notably, treatment of adult wild-type mice with ER antagonists, fulvestrant or raloxifene, also caused a significant depletion of the oocyte pool, although this depleted oocyte pool spontaneously rebuilt to its normal size after the cessation of ER antagonist treatment. Thus, through both genetic and pharmacological approaches, E2 signaling appears to play a crucial role in mediating OSC differentiation and oocyte renewal in adult mouse ovaries.
Collectively, our findings uncover a dynamic role for steroid hormone signaling in regulating mammalian OSC activity and consequently the size of oocyte pool during female reproductive cycle. Of immediate clinical relevance, these studies suggest that the potential effects of ER modulators, such as selective estrogen receptor modulators (SERMs) which are received by patients worldwide for various therapeutic purposes, on OSC function and the ovarian oocyte reserve should be considered.

W-2431

TARGETED ABLATION AND LINEAGE TRACING MOUSE MODELS CONFIRM OOCYTE RENEWAL IN POSTNATAL MAMMALIAN OVARIES

Ning Wang, Chonthicha Satirapod, Jonathan L. Tilly
Obstetrics and Gynecology, Massachusetts General Hospital, Boston, MA, United States

Contrary to the common belief that mammalian females are born with a fixed pool of oocytes, past studies in mice show that oocyte numbers remain remarkably stable during young adulthood despite of a large number of oocytes being removed by atresia and ovulation after growth activation. This suggests the possible existence of an active oocyte renewal process in adult ovaries that may involve germline stem cells (GSCs). Such a notion is further bolstered by the identification and recent isolation of a rare population of mitotically active germ cells in ovaries of adult female mice and of reproductive age women. These putative GSCs, or oogonial stem cells (OSCs), possess the ability, at least in mice, to generate functional eggs upon transplantation into recipient ovaries. However, experimental evidence supporting oocyte renewal from precursor cells, e.g., OSCs or their progeny, under normal physiological conditions is missing.

Meiosis can be viewed as a stem cell differentiation process unique to the germ line. *Stimulated by retinoic acid gene 8* (*Stra8*) is a germ cell-specific gene transiently expressed during a narrow window of time associated with meiotic initiation in differentiating GSC progeny of both sexes. *Stra8* is absent in undifferentiated GSCs and in mature germ cells such as sperm and oocytes. Recently, we showed that *Stra8* activation occurs in adult ovaries and often precedes increasing oocyte numbers, suggesting that OSCs, like other GSCs, activate *Stra8* to enter meiosis. This information has allowed us to develop a novel suicide gene transgenic (sg-Tg) mouse line in which the *Stra8* promoter drives expression of *Herpes simplex virus thymidine kinase* (*HSVtk*). In this line, Stra8+ germ cells generated by OSCs to produce new oocytes can be selectively ablated by exposure to ganciclovir (GCV). We show that the oocyte pool in ovaries of adult sg-Tg mice declined 44% after treating mice with GCV for 21 days, representing a loss of around 80 oocytes not being added to each ovary per day. OSCs do not express *Stra8* and were intact during GCV treatment. Thus, once the GCV-based ablation of Stra8+ germ cells was ceased, oocyte numbers spontaneously regenerated to their normal level as oogenesis resumes from OSCs. Importantly, this reversible oogenic failure in adult ovaries paralleled an expected reversible spermatogenic failure in adult testes when Stra8+ germ cells were temporally ablated using GCV. Interestingly, although OSCs are still present in aged ovaries, ablation of Stra8+ germ cells did not cause a change in oocyte numbers in older mice, indicating an absence of oocyte renewal during ovarian aging. To confirm these findings, we also developed an inducible lineage-tracing mouse model (*Stra8-R26R*) to genetically and permanently label with YFP any new oocytes being formed from OSCs during doxycycline (Dox) induction. After feeding adult female *Stra8-R26R* mice with Dox for 21 days, we observed a chimeric pool of follicles containing both YFP+ and YFP-negative oocytes. In addition, follicles containing YFP+ oocytes were observed at both resting and growing stages, indicating adult-derived oocytes mature normally.

Taken together, our studies firmly establish new oocyte production from OSCs undergoing meiotic commitment through *Stra8* activation, and demonstrate that oocyte renewal is required for the maintenance of the adult ovarian reserve in mammals. Our data also indicate that a progressive decline of OSC activity contributes to oocyte loss and ovarian aging.

W-2432

RENAL TUBULAR LIKE CELLS GENERATED FROM MURINE GERMLINE CELL DERIVED PLURIPOTENT STEM CELLS PROTECT KIDNEY FROM ISCHEMIC INJURY

Letizia De Chiara[1], Sharmila Fagonee[1], Andrea Ranghino[2], Stefania Bruno[1], Giovanni Camussi[2], Emanuela Tolosano[1], Lorenzo Silengo[1], Fiorella Altruda[1]

[1]Department of molecular biotechnology and health sciences, Molecular Biotechnology Center, Torino, Italy, [2]Department of Internal Medicine, University of Turin, Torino, Italy

Spermatogonial Stem Cells are unipotent stem cells located on the basal membrane of the testis and continuously generate differentiating daughter cells for production of haploid spermatozoa. In vitro Spermatogonial Stem Cells are able to switch to pluripotent cells, the Germline-cell Derived Pluripotent Stem Cells (GPSCs). The conversion toward pluripotent state occurs spontaneously without transduction of reprogramming factors. GPSCs share many features with Embryonic Stem Cells, as are able to self-renew and differentiate into derivatives of all three germ layers. Moreover, GPSCs have a great plasticity, being able to differentiate in vitro into functional cardiomyocytes, neurons and hepatocytes. To investigate if GPSCs can be differentiate into renal tubular cells we devised a new

protocol to induce renal epithelial differentiation and successfully obtained GPSCs derived tubular-like cells (GTCs). GTCs were functional in vitro as demonstrated through Transepithelial Electrical Resistence (TEER) analysis. To investigate whether GTCs could protect against renal failure we studied a model of kidney ischemia/reperfusion injury. Soon after ischemic damage mice were injected with GTCs (treated group) or vehicle (control group). We found that GTCs were able to home to renal parenchyma. GTCs-treated mice showed reduced oxidative stress, apoptosis and cortical damage associated to a significant up-regulation of the anti-oxidant enzyme heme oxygenase (HO)-1.Mice treated with GTCs were also protected from chronic renal disease development as demonstrated by lack of fibrosis and decreased inflammatory infiltrate six weeks after ischemic injury.
In conclusion, we report for the first time that GPSCs can be differentiated into renal tubular-like cells and that these cells are functionally active in vivo protecting kidney from ischemic damage. This finding demonstrates the great plasticity of GPSCs and open the possibility to take advantage of GPSCs in regenerative cell therapy.

W-2433

ANALYSIS OF HUMAN PARTHENOGENETIC IPS CELLS IDENTIFIES A NOVEL REGULATION OF ONCO-MICRORNA-371-3 IN TESTICULAR GERM CELL TUMORS

Yonatan Stelzer, Ido Sagi, Nissim Benvenisty
Genetics, The Hebrew University of Jerusalem, Jerusalem, Israel

Parental imprinting is a form of parent-of-origin epigenetic marking inherited from the gametes, which restricts asexual reproduction in placental mammals. Imprinted genes are expressed in a monoallelic fashion thus rendering them highly susceptible to genetic and epigenetic perturbations, potentially resulting in transformation and disease. Indeed, loss-of-imprinting in several genes (e.g., H19, IGF2 and MEST) has been correlated with different types of malignancies such as Wilm's tumor, colorectal neoplasia and lung cancer. Nevertheless, the causative role of imprinting in tumorigenesis is still poorly understood, so that the precise mechanisms by which imprinted genes might contribute to tumorigenicity remain to be elucidated. In this study we demonstrate that the onco-microRNA-371-3 are highly expressed in our established human parthenogenetic iPS cell lines, which harbor only maternal alleles. RNA deep sequencing analysis enabled the identification of a novel paternally expressed gene, transcribed in an antisense orientation to the onco-microRNA-371-3. We further demonstrated that the antisense transcript inhibits the expression of its corresponding microRNAs, thus indicating to a novel form of microRNA regulation in the human genome. As microRNA-372-3 were shown to play an oncogenic role in testicular germ cell tumors, we studied the involvement of its antisense transcript in these cells. Our results suggest that hypermethylation leading to loss-of-expression of the imprinted antisense transcript, contributes to tumorigenic transformation by affecting down-stream targets such as LArge Tumor Suppressor homolog 2 (LATS2). Furthermore, we could demonstrate that the novel imprinted transcript may play a tumor suppressive role, as its over-expression in tumor cells results in cell growth arrest and apoptosis. In conclusion, we report the involvement of a novel imprinted gene in testicular germ cell tumors, thus demonstrating the potential effect of loss-of-imprinting on tumor transformation. We also describe a novel form of microRNAs regulation in the human genome, suggesting a complex cross-talk in cancer and early embryogenesis between oncogenic-microRNAs and their tumor-suppressor antisense transcript.
* The first part of this research was recently published (See Stelzer et al. Nature Structural and Molecular Biology, 2011)

Pre-clinical and Clinical Applications of Mesenchymal

W-3001

HUMAN UMBILICAL CORD BLOOD-DERIVED MESENCHYMAL STEM CELLS EXPRESSING BDNF ENDOGENOUS NEUROGENESIS IN AN ISCEHMIC STROKE MODEL

Changhyun Jeong, Seong Muk Kim, Chung Heon Ryu, Sin-Soo Jeun
The Catholic University of Korea, Seoul, Korea, Republic of

Numerous studies have reported that mesenchymal stem cells (MSCs) can ameliorate neurological deficits in ischemic stroke models. Among the various hypotheses that have been suggested to explain the therapeutic mechanism underlying these observations, neurogenesis is thought to be critical. To enhance the therapeutic benefits of human umbilical cord blood-derived MSCs (hUCB-MSCs), we efficiently modified hUCB-MSCs by introduction of the brain-derived neurotrophic factor (*BDNF*) gene via adenoviral transduction mediated by cell-permeable peptides, and investigated whether *BDNF*-modified hUCB-MSCs (MSCs-BDNF) contributed to functional recovery and endogenous neurogenesis in a rat model of middle cerebral artery occlusion (MCAO). Transplantation of MSCs induced the proliferation of 5-bromo-2'-deoxyuridine (BrdU)-positive cells in the subventricular zone. Transplantation of MSCs-BDNF enhanced the proliferation of endogenous neural stem cells more significantly, while suppressing cell death. Newborn cells differentiated into doublecortin (DCX)-positive neuroblasts and Neuronal Nuclei (NeuN)-positive mature neurons in the subventricular zone and ischemic boundary at higher rates in animals with MSCs-BDNF compared with treatment using solely phosphate buffered saline (PBS) or MSCs. Triphenyltetrazolium chloride staining and behavioral analysis revealed greater functional recovery in animals with MSCs-BDNF compared with the other groups. MSCs-BDNF exhibited effective therapeutic potential by protecting cell from apoptotic death and enhancing endogenous neurogenesis.

W-3002

SERUM STARVED ADIPOSE DERIVED STROMAL CELLS AMELIORATE RAT CRESCENTIC GLOMERULONEPHRITIS BY PROMOTING IMMUNOREGULATORY MACROPHAGES

Kazuhiro Furuhashi, Naotake Tsuboi, Yiqin Shi, Hangsoo Kim, Takayuki Katsuno, Takenori Ozaki, Seiichi Matsuo, Shoichi Maruyama
Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan

Introduction: Glomerular crescents determine renal prognosis in patients with rapidly progressive glomerulonephritis, however efficient therapeutic approach to regulate crescent formation has not emerged since diverse leukocyte subsets contribute to the lesion. We have reported that adipose
tissue-derived stem cells (ASC) promoted regeneration in a rat model of acute kidney injury. More recently, we have shown that ASC more strongly modulate T-cell immune reaction than bone marrow derived mesenchymal stem cells (BM-MSC). In the present study, we examined the renal protective effects of ASC focusing on their immunomodulatory properties in anti-glomerular basement membrane glomerulonephritis (anti-GBM GN) leading severe crescentic formation.
Methods: Necrotizing crescentic glomerulonephritis was induced in WKY rats by intraperitoneal injection of anti -GBM mAb, which closely resembles to nephritis in human Goodpasture disease. Renal function and histology were assessed in animals treated with ASC or BM-MSC. To evaluate ASC-driven functional M2 polarization in macrophage, we cultured peritoneal macrophages with ASCs or BM-MSC. For the evaluation of LASC-derived soluble factors in macrophage M2 conversion, PGE2 synthesis and IL-6 signaling in co-culture medium of macrophages with LASC were inhibited by pharmaceutical compounds or neutralizing antibody for respective receptors.
Results: ASC administered systemically to rats had a significant preventive effect against renal injury that was accompanied by a considerable decrease in proteinuria, crescent formation, and infiltration by glomerular leukocytes, including neutrophils, CD8+ T cells, and CD68+ macrophages. Interestingly, LASC increased the number of glomerular CD163+ macrophages known as immunoregulatory M2 macrophages, while this effect was not observed in BM-MSC. IL-10 concentration in renal cortex from diseased rat was higher in ASC group than in control group. In vitro co-culture system clearly demonstrated that ASC, but not BM-MSC, directly turned macrophage into M2 phenotype. Moreover, these effects of ASC were more prominent in LASC than HASC. Administrated ASCs were scattered in multiple organs and number of ASC in diseased glomeruli was 1.5/glomerulus/cross section without any difference between HASC and LASC, but this would be efficient since individual LASC could polarize around 200 macrophages to M2 phenotype in vitro. These results suggest that LASC recruited into diseased glomerulus make stronger effect on macrophages for protection of glomerular injury and crescent formation than HASC. Moreover pharmaceutical ablation of PGE2 production or neutralization of IL-6 in the co-culture medium significantly reversed LASC-mediated macrophage conversion.

Conclusion: We found that LASC-derived IL-6 and PGE2 induce CD163+ macrophages in our *in vitro* co-culture system. ASC exerted profound immunoregulatory properties especially on macrophages and ameliorated glomerular injury in rat anti-GBM glomerulonephritis model. In human immunosuppressive therapy such as corticosteroid and cyclophosphamide remains the prevailing therapy for CGN, but this is often restricted for their adverse effect such as infection and agent cytotoxicity. Therefore, LASC administration would be desirable therapeutic approach to improve prognosis of anti-GBM GN patients.

W-3003

MESENCHYMAL STEM CELL THERAPY AMELIORATES DEXTRAN SODIUM SULFATE-INDUCED COLITIS IN MICE BY REGULATING BONE MARROW NICHE CELLS AND HEMATOPOIETIC STEM CELLS

Kanna Nagaishi[1], Hiroyuki Isshiki[1], Yoshiaki Arimura[1], Yasuyoshi Naishiro[1], Mineko Fujimiya[1], Kozoh Imai[2], Ysuhisa Shinomura[1]

[1]Sapporo Medical University, Sapporo, Japan, [2]Tokyo University, Tokyo, Japan

Background/Aim: Two major functions of mesenchymal stem cells (MSCs) are repair and regeneration of target tissue and immune-modulatory function through production of trophic factors, cell supplementation, and cell-cell interaction. We have shown that MSC therapy reduced the severity of colitis in mice. In spite of only few numbers of MSCs distributed in colonic tissue, MSCs were engrafted in bone marrow and its amount seemed to be amplified. Hematopoietic stem cells (HSC) are known to differentiate into mature hematopoietic cells, which are source of inflammatory cells, and its quiescence is regulated by niche cells in bone marrow. The most quiescent HSC known as long term reconstitute HSC (LT-HSC) are cross-talking with niche cells by abundant molecules and maintain its functions. In this study, we investigated whether the hyper-cytokinemia in colitis could induce niche cell perturbation, whether abnormal niche cells secondary affected HSCs to provide excessive mature inflammatory cells in colonic tissues, and whether MSC therapy could reverse niche cell perturbation and subsequent abnormality of HSCs in bone marrow.
Methods: MSCs were administered to dextran sodium sulfate (DSS)-induced colitis mice. Therapeutic effects of MSCs were analyzed by pathological findings of colonic tissues. Kinetics and molecular profile of Nestin-positive niche cells (Nes^+ cells) in bone marrow of control mice, DSS colitis-induced (DSS) mice and MSC-treated DSS colitis-induced (DSS-MSC) mice were investigated. HSCs were fractionated by the differentiation and analyzed the expression of functional molecules. Nes^+ cells isolated from control mice and DSS mice were co-cultured with LT-HSC derived from DSS mice respectively to clarify the effect of niche cell replacement for the abnormality of HSCs. Furthermore, niche cells isolated from DSS mice were cultured with or without MSCs *in vitro* to investigate the effect of MSCs to abnormal niche cells.
Results; The number of Nes^+ cells in bone marrow and colony forming unit ability were remarkably reduced in colitis mice and were recovered with MSC therapy. Expression of Wnt5a, stem cell factor (SCF) and stromal cell-derived factor-1 (SDF1) in Nes^+ cells were remarkably decreased in DSS mice, while the reduction was prevented in DSS-MSC mice. Expression of CXCR4 in LT-HSC was reduced in DSS mice compared to control mice, which were up-regulated by replacement of abnormal niche cells *in vitro*. This result was similar to the

expression profile of LT-HSCs in DSS mice and control mice *in vivo*. Expression of Osteopontin in Nes^+ cells was reduced
in DSS mice compared to control mice, which were recovered by exposed to MSCs *in vitro*. This result was similar to the
expression profile of Nes^+ cells in DSS mice and control mice *in vivo*. Finally, expressions of frizzled family receptor 4 (Fzd4), beta-catenin and Tie2 in LT-HSCs were extremely down-regulated in DSS mice and were prevented in DSS-MSC mice.
Conclusion: HSCs are supported by niche cells by various factors, such as Wnt signaling, adhesion molecule, chemokines and growth factors. These are down-regulated in colitis and affected to the interaction with HSCs, which resulted in excessive supply of activated inflammatory cells in colonic tissue by losing quiescence and excessive mobilization from niche. MSC therapy has an ability to suppress severe colitis via regulation of niche function in the bone marrow. These findings may provide the new target of IBD therapy.

W-3004

MESENCHYMAL STEM CELLS HOME TO OCULAR SURFACE AND SUPPRESS ALLOIMMUNITY IN CORNEAL TRANSPLANTATION

Sunil Chauhan, Masahiro Omoto, Reza Dana
Department of Ophthalmology, Harvard Medical School, Schepens Eye Research Institute, Massachusetts Eye and Ear, Boston, MA, United States

Purpose: To investigate whether systemically-injected host mesenchymal stem cell (MSC) can home to the transplanted cornea, suppress induction of alloimmunity, and promote allograft survival.
Methods: MSC ($CD45^-CD34^-SCA1+CD29+$) were generated from the bone marrow of wild-type BALB/c or GFP+ C57BL/6 mice, and $1x10^6$ cells were intravenously injected to allografted recipients 2h after surgery. To track the homing of GFP+ MSC (C57BL/6), corneal grafts from BALB/c (H-2d) mice were transplanted onto C57BL/6 (H-2b) recipient mice. MSC homing to the corneas was examined at day 3 post-transplantation by immunohistochemistry. To investigate the effect of MSC on alloimmunity and graft survival, corneal grafts from C57BL/6 mice were transplanted onto BALB/c recipient mice, and then wild-type BALB/c MSC were injected. Frequencies of alloreactive IFNγ+ T cells were analyzed at day 14 post-transplantation using the ELISPOT assay. Frequencies of mature CD11C+MHC-II+ antigen-presenting cells (APC) were analyzed by flow cytometry. Graft survival was evaluated by slit-lamp biomicroscopy weekly up to 8 weeks.
Results: Intravenously injected GFP+MSC were found in abundance in the transplanted cornea, but not in the ungrafted (contralateral) cornea. The frequencies of mature CD11C+MHC-II+ antigen-presenting cells were substantially decreased in the corneas (50.2% vs. 76.7%) and draining lymph nodes (4.4% vs. 8.4%) of MSC-injected allograft recipients compared to control group. The draining LN of MSC-injected allograft recipients showed significantly lower frequencies allosensitized IFNγ-secreting T cells compared to the control group (p=0.023). Allograft survival rate was significantly (~2-fold) higher (p = 0.03) in the MSC-injected recipients (80%, n=12) compared to the non-MSC injected group (40%, n =10).
Conclusion: Our data demonstrate that systemically-administered MSC specifically home to transplanted corneas and promote allograft survival by inhibiting APC maturation and induction of alloreactive T cells. These data suggest that host MSC exert immunomodulatory functions in corneal transplantation and may be used to prolong transplant survival.

W-3006

BASIC STUDIES USING BONE MARROW-DERIVED MESENCHYMAL STEM CELLS FOR THE DEVELOPMENT OF A LESS INVASIVE LIVER REGENERATION THERAPY FOR LIVER CIRRHOTIC PATIENTS

Taro Takami[1], Shuji Terai[1], Luiz Fernando Quintanilha de Mesquita[1], Haruko Tanimoto[1], Koichi Fujisawa[2], Naoki Yamamoto[3], Isao Sakaida[1]

[A]Department of Gastroenterology and Hepatology, [B]Center for Reparative Medicine, [1]Yamaguchi University Graduate School of Medicine, Ube, Yamaguchi, Japan, [2]Yamaguchi University Health Administration Center, Yamaguchi University, Yamaguchi, Yamaguchi, Japan

Background and Objectives: We have developed an autologous bone marrow cell infusion (ABM*i*) therapy that is a safe and efficient liver regeneration therapy for liver cirrhotic patients using non-cultured autologous whole bone marrow (BM) cells. However, this therapy involves BM aspiration under general anesthesia and is not indicated in patients for whom general anesthesia must be avoided. We have therefore started to develop a less invasive liver regeneration therapy that uses autologous BM-derived mesenchymal stem cells (BM-MSCs) and requires small amounts of autologous BM aspirated under local anesthesia. Recently, it has been demonstrated that MSCs show antioxidant activity, which may be important in the treatment of liver diseases, as oxidative stress plays an important role in liver disease development. Here, we attempted to test MSCs in liver regeneration and reveal the underlying mechanisms, particularly with regard to antioxidant activity.
Methods: Cultured BM-MSCs were analyzed based on morphology, flow cytometry and differentiation protocols. To assess effects of these cells on liver injury in vivo, we systemically infused BM-MSCs into NOD-SCID cirrhotic mice. Liver fibrosis and hepatic stellate cell (HSC) activation were assessed by Sirius red staining and immunohistochemistry, respectively. Injury serum markers such as **alanine transaminase (ALT), aspartate transaminase (AST) and lactate dehydrogenase (LDH), beyond total antioxidant activity were evaluated.** To confirm the protective effects against oxidant conditions, we used hydrogen peroxide (H_2O_2) or thioacetamide (TAA) in order to induce oxidative stress conditions in vitro. We then quantified cellular reactive oxygen species (ROS) and enzymes related to oxidative stress resolution in HSCs and hepatocytes co-cultured with BM-MSCs.
Results: We confirmed that our cells showed MSC characteristics. They were adherent to plastic, positive for CD73/CD90/CD105 and negative for CD45/CD11b, and were able to differentiate into adipocytes and osteocytes. In NOD-SCID mice, they were able to reduce fibrosis ($p<0.05$) and hepatic αSMA-positive area ($p<0.05$). In addition, mice treated with BM-MSCs showed lower levels of ALT ($p<0.05$) and AST ($p<0.05$), and higher total antioxidant activity ($p<0.001$). HSCs co-cultured with BM-MSCs showed lower levels of ROS-signals ($p<0.05$), consistent with up-regulated expression of heme oxygenase-1 ($p<0.01$) and superoxide dismutase-3 ($p<0.001$). Moreover, we confirmed that hepatocytes co-cultured with MSCs had lower levels of ROS ($p<0.001$), and higher expression of NF-E2-related factor 2 ($p<0.05$) and glutathione *S*-transferase ($p<0.01$).
Conclusions: These results demonstrate that infused BM-MSCs are involved in the improvement of liver injury through stabilization of redox homeostasis, which strongly indicates the possibility of a less invasive liver regeneration therapy for liver cirrhotic
patients based on infusion(s) of BM-MSCs.

W-3007

BAROREFLEX SENSITIVITY AND CARDIAC FUNCTION IN RATS WITH HEART FAILURE TRANSPLANTED WITH ALLOGENEIC MESENCHYMAL STEM CELLS.

Sharon D B V Morais[1], Renata M. Lataro[1], Carlos Alberto Aguiar Silva[1], Luciano F L Oliveira[1], Eduardo E V Carvalho[1], Christiane Becari[1], Marcus Vinicius Simões[1], Lindolfo da Silva Meirelles[2], Hélio Cesar Salgado[1], Rubens Fazan Jr.[1]

[1]Physiology, Medicine School of Ribeirão Preto / University of São Paulo, Ribeirão Preto, Brazil, [2]Lutheran University of Brazil, Canoas, Brazil

Background. Patients with heart failure induced by myocardial infarct (MI) have impaired baroreflex function, which is an important risk for life threatening. The baroreflex plays an important modulatory role on heart rate

(HR), cardiac output, myocardial contractility and regional distribution of blood flow. Therapy with mesenchymal stem cells (MSC) has shown promising results after MI in either patients or experimental animals, showing pro-angiogenic, anti-fibrotic and immunomodulatory effects. The aim of this study was to evaluate the effect of MSC on baroreflex sensitivity and cardiac function in rats with heart failure induced by MI.
Methods and Results. Male Wistar rats (250-320g) were treated, intravenously, with MSC (N=4) or saline (N=5) 1 week after surgical ligation of left coronary artery. Sham-operated (non-infarcted, N=5) control rats were also evaluated. Transplanted MSC were characterized according to criteria of the International Society for Cellular Therapy. The size of MI was evaluated by single photon emission computed tomography (SPECT) 2-3 days after coronary ligation and were found similar among infarcted rats (51±1%). Heart function (left ventricle ejection fraction) was assessed by isotopic cardiac ventriculography before and one month after administration of MSC. After the second ventriculography, animals underwent 12 lead electrocardiographic recording and were implanted with catheters into femoral artery and vein. After 24h of vessel catheterization, conscious freely moving rats had their basal arterial pressure (AP) and HR recorded. Following, phenylephrine (8 μg/kg, iv) was used to induce an acute rise in AP and baroreflex mediated fall in HR. The ratio between changes in HR and AP was used as an index of baroreflex sensitivity. At the end of the experiments, the animals were killed and the hearts were collected for histopathological analysis. The extension of the infarct was found smaller in rats treated with MSC (36±3%) as compared with the saline treated counterparts (47±2%). Ventricular performance was markedly impaired after MI (42±3.4% control, 15±2.4% before saline injection and 17±1.9% before MSC injection), but MSC did not improve cardiac function (51±2% control, 16±1% saline and 20±4% MSC). Interstitial collagen was 10±1% in sham operated control rats. Infarcted rats showed an increase in interstitial collagen (15±2%), that was not observed in those treated with MSC (11±1%). Baroreflex sensitivity was lower after MI (1.10±0.1 bmp/mmHg); nevertheless, infarcted rats that received MSC presented baroreflex sensitivity similar to non-infarcted control rats (1.74±0.2 vs. 1.80±2 bpm/mmHg). QT and QTc intervals were lengthened by MI, while MSC did not affect this parameter (iQT: 85 ± 2.4ms control vs. 97 ± 1.4ms saline and 97 ± 0.8ms MSC; iQTc: 197 ± 3.2ms control vs. 228 ± 6.8ms saline and 223 ± 4.5ms MSC).
Conclusion: Altogether, the results show, after one month of treatment, that MSC therapy reduced the extension of the infarction and the interstitial fibrosis in the surviving myocardial of the left ventricle, besides improving baroreflex sensitivity.

W-3008

WEEKLY INTRAARTICULAR INJECTIONS OF SYNOVIAL MESENCHYMAL STEM CELLS DELAY CARTILAGE DEGENERATION THROUGH TROPHIC FACTORS IN A RAT OSTEOARTHRITIS MODEL

Nobutake Ozeki[1], Ichiro Sekiya[2], Kunikazu Tsuji[3], Tomoyuki Saito[4], Takeshi Muneta[1]
[A]Department of Joint Surgery and Sports Medicine, [B]Department of Cartilage Regeneration, [C]Global Center of Excellence Program, [1]Tokyo Medical and Dental University, Tokyo, Japan, [2]Department of Orthopaedic Surgery, Yokohama City University, Yokohama, Japan

INTRODUCTION
Osteoarthritis is a degenerative joint disease associated with cartilage breakdown and inflammation of synovium. We previously reported that mesenchymal stem cells (MSCs) derived from synovium had a high proliferation and chondrogenic potential, and transplantation of synovial MSCs into a full-thickness cartilage defect promoted cartilage regeneration. In this study, we investigated whether intraarticular injections of synovial MSCs delayed cartilage degeneration in a rat osteoarthritis model.
METHODS
Animals and surgery: Wild type male Lewis rats at 11-13 weeks were used. To induce osteoarthritis, anterior cruciate ligament was transected.
Weekly injections of synovial MSCs: From 1 week after the surgery, 1 x 10^6 syngenic synovial MSCs were injected into the knee joint every week (weekly group). The one shot group had only one injection of MSCs, and the control group had weekly injections of PBS alone. Both femoral and tibial cartilage were evaluated macroscopically and histologically at 5, 9, and 13 weeks after the surgery.

In vivo bioluminescent imaging (IVIS): To chase the injected MSCs, IVIS was used to detect photons from synovial MSCs derived from luciferase transgenic rats.
Detection of LacZ expression: To clarify cell distribution of injected cells, X-Gal staining was evaluated after injection of synovial MSCs derived from LacZ transgenic rats ($LacZ^+$ MSCs).
Quantitative measure and gene expression of the engrafted synovial MSCs: For a quantitative measure of the engrafted synovial MSCs, synovium of human MSCs(hMSCs) injected samples were compared to control by a quantitative RT-PCR assay specific for human β-actin. Control samples were prepared by adding various numbers of hMSCs to the synovium before homogenizing, extracted RNA. Additionally, gene expression of rat and human RNA was evaluated.
RESULTS
Weekly injections of synovial MSCs delayed cartilage degeneration. Macroscopic and histological observations for cartilages revealed cartilage lesions gradually progressed in the one shot group and in the control group, while cartilages in the weekly group appeared to be better throughout the study. The OARSI score for cartilage histopathology was significantly better in the weekly group at 9, and 13 weeks than in the other groups. Vascular formation in the infrapatellar fat pad increased in the control group, but it did not in the weekly group.
MSCs could be detected in the knee joint at all times when injected weekly. After a one shot injection, IVIS showed MSC-derived photons detected around the knee decreased within 14 days. When MSCs were injected weekly, they could be detected at all times.
Most MSCs adhered to the synovium and expressed TSG-6. One day after injection of $LacZ^+$ MSCs, dark blue areas for LacZ were observed mainly in the synovium macroscopically, and LacZ-positive cells could be confirmed in the synovium histologically. PCR analysis revealed that about 1 % of injected MSCs survived within synovium 1 day after injection, and expression of human TSG-6 was upregulated.
DISCUSSION
Weekly intraarticular injections of synovial MSCs delayed cartilage degeneration in a rat osteoarthritis model. One of the mechanisms to delay progression of osteoarthritis is considered to be due to the trophic effect of MSCs to suppress synovial inflammation. After injection of MSCs into the knee joint, they rapidly decreased. As osteoarthritis is a chronic and progressive disease, not a single injection, but periodical injections are recommended.

W-3011

EXPOSURE OF MESENCHYMAL STEM CELLS OR CULTURED PROXIMAL TUBULE STEM CELLS TO A KIDNEY INJURY ENVIRONMENT INDUCES OXIDATIVE AND MITOCHONDRIAL STRESS

Jon D. Ahlstrom[1], Florian E. Toegel[2], Zhuma Hu[1], Ping Zhang[1], Christof Westenfelder[3]
[1]Medicine, VA medical center, Salt Lake City, UT, United States, [2]Nephrology, Harvard Medical School, Boston, MA, United States, [3]Physiology, University of Utah, Salt Lake City, UT, United States

Acute kidney injury (AKI) is a common and largely treatment-resistant syndrome in which complex mechanisms mediate injury and repair. Pre-clinical studies from our laboratory have led to a Phase I Clinical Trial in which Mesenchymal Stem Cells (MSCs) were administered to at-risk patients undergoing cardio-pulmonary bypass surgery to test the safety and preliminary efficacy of MSCs for the prevention of post-op AKI. It is well recognized that the changes in the “milieu interne” that result as a consequence of AKI adversely affect the function of distant organs in addition to the kidney. Hence, it is possible that the AKI environment into which the MSCs are placed in vivo could be harmful to the cells administered as a therapy. The objective of this present study was to explore the consequences of AKI on the cellular responses of MSCs, using a model of culturing rat MSCs or rat kidney cells (NRK cell line), in serum from rats with experimentally induced AKI. To confirm the relevance of our assay, we also compared our in vitro results with cells sampled from tissues and organs in vivo following AKI.
Exposure of MSCs or NRK cells to 10% AKI serum for 48 hours caused, compared to incubation with SHAM serum or animal sera obtained following nephrectomy (NPHX), the upregulation of anti-oxidant genes (catalase, HO-1), increased reactive oxygen species activity (CMH2DCFDA), higher mitochondrial membrane potential (TMRE), increased mitochondrial complex I activity, and reduced mitochondrial reserve capacity (Seahorse XF). Tissues collec-

ted from organs in vivo, such as the kidney, heart and lung, also showed a similar response, with increased HO-1 gene expression and higher mitochondrial membrane potential following AKI.
Summary: Exposure of MSCs to a kidney injury environment resulted in greater cellular oxidative and mitochondrial stress. This result required the presence of injured tissue, because at comparable levels of kidney dysfunction (AKI vs. NPHX), the stressful conditions were found in the AKI, and not the SHAM or NPHX environment. This indicates that the injured kidney per se generates signals that are traumatic for MSCs and other cells types.
In conclusion: These data identify yet inadequately understood pathogenic signals that are generated by the injured kidney, and whose nature, once defined, is expected to further advance our understanding of the acutely uremic state and how it affects renal and systemic cell biology and organ functions. In addition, these initial observations provide the basis for the development of therapeutic interventions that are both renoprotective and can overcome the negative effects of AKI.

W-3012

IMMUNOMODULATORY EFFECTS OF FETAL MESENCHYMAL STEM CELLS

Julie SL Yeo[1], Jing Yao Leong[3], Joo Guan Yeo[3], Mark SK Chong[1], Jinhua Lu[2], Jia Lu[5], Mahesh A. Choolani[1], Jerry KY Chan[4]

[A]Department of Obstetrics & Gynaecology, [B]Department of Microbiology, [1]National University of Singapore, Singapore, Singapore, [A]Children's Intensive Care Unit, [B]Department of Reproductive Medicine, [2]KK Women's and Children's Hospital, Singapore, Singapore, [3]Combat Performance Lab, DSO National Laboratories, Singapore, Singapore

Introduction
The immunomodulatory properties of Mesenchymal Stem Cells (MSC) are currently under investigation for their potential to treat autoinflammatory conditions. This includes acute lung injury (ALI), where, following a minimally traumatic insult, a rapid influx of inflammatory cells is accompanied by an increase in pro-inflammatory cytokines leading to disruption of the alveolar capillary interface, and ultimately resulting in alveolar oedema and respiratory failure. MSCs have been proposed for the management of ALI, where, following lung injury, administered MSC may arrest the progression of the inflammatory cascade and thus prevent the degeneration of lung function. Here, we hypothesised fetal MSC (fMSC) to attenuate inflammatory reactions of macrophages, and explored their utility in a murine model of smoke-induced ALI.
Methods
Following informed consent, fetal femurs were collected for isolation of fMSC after clinically-indicated termination of pregnancy. fMSC were characterised according to ISCT guidelines. An in vitro macrophage inflammatory assay was used to study the immonomodulatory mechanism of fMSC. Monocytes were derived from healthy human blood samples and exposure to Granulocyte-macrophage colony-stimulating factor (GM-CSF to yield macrophages. The macrophages were stimulated with LPS and fMSC were subsequently added and cytokine expression of IL-6, IL-8 and TNF-alpha were determined after 24h using ELISA. For evaluation of utility in the treatment of ALI, an immunocompetent murine model of ALI by smoke inhalation was generated. Lung injury at 24h was characterised by bronchio-alveolar lavage (BAL) cell counts and protein concentration, wet/dry ratio, cytokines (IL-6, MCP-1, KC, G-CSF and IP-10) and myeloperoxidase (MPO) analysis on lung tissues. Histological analysis of lung tissue was conducted to assess extent of lung damage. Treatment with 0.5x10^6 cells (cell passages 4-6) was introduced intratracheally between 1-2hr after smoke inhalation injury. BAL and lung tissue were collected at 24h and assayed for various lung injury parameters.
Results
fMSC incubated with stimulated macrophages demonstrated two-fold reduced TNF-alpha levels with no significant changes observed in IL-6 and IL-8 levels. These findings could be reproduced in a transwell set-up but not with MSC-conditioned medium, suggesting a primarily paracrine signalling mechanism with crosstalk. A murine model of smoke inhalation-induced ALI was generated, demonstrating increase in BAL cell counts, wet/dry ratio, cytokines and MPO as well as an increase in the histological lung injury index ($p<0.05$).Following treatment with MSC, reduc-

tion in BAL cell counts (4.1x10^5 vs 3.2x10^5 cells), protein concentration (198ug/ml vs 151ug/ml) and cytokine (G-CSF, IP-10) levels were observed.
Conclusion
The immunomodulatory properties of fMSC on inflammatory cells were studied, and shown to suppress TNF-alpha levels in a paracrine fashion. Thus, following administration to a murine model of smoke inhalation-induced ALI, fMSC were able to reduce lung injury. Taken together, our results suggest fMSC to exhibit anti-inflammatory properties, with potential applications in the management of autoinflammatory conditions.

W-3013

MSC REGENERATIVE POTENTIAL; DOES DONOR AGE MATTER

Martha L. Arango, Paulette A. Conget
Facultad de medicina, Universidad Del Desarrollo, Santiago de Chile, Chile

In pre-clinical and clinical studies, it has been proved that the transplantation of multipotent mesenchymal stromal cells, also referred as to mesenchymal stem cells (MSCs), results in therapeutic effect. Currently there is no systematic information regarding whether donor age modify the regenerative potential of MSCs. Nevertheless, the following age-related changes have been shown: decline in the abundance, increase in senescent cell frequency, defeat of proliferation potential, reduction of immunomodulatory potential, and loss of in vivo bone formation potential.
Our aim was to compare the regenerative potential of syngeneic MSCs obtained from young (8-weeks-old), middle age (33-weeks-old) or elder (50-weeks-old) female donors. For this, two doses of MSCs (0.5 or 1x106) or two doses of medium conditioned by MSCs (1x or 6x) were administered subcutaneously around full thickness excisional splits of 6 mm diameter performed in the midline of 8-weeks-old male C57BL6 mice. Every two days after MSC transplantation, wound healing was assessed macroscopically (wound closure, retraction and scare formation) and microscopically (re-epithelialization, dermal-epidermal junction formation, granulation tissue formation, leukocyte infiltration, fibrosis and skin appendage structure regeneration).
As expected, wound closure was accelerated in cutaneous lesions that received MSCs or their conditioned medium, compared with lesions that received only the vehicle (8 vs. 12 days, respectively). Surprisingly, we found that MSCs and their conditioned medium obtained from young donors were the less efficient tools to fasten wound closure (50% at day 6-8 for young vs. day 4-5 for middle and elder). This difference was not overcome by increasing cell or medium doses. Also, we observed the administration of MSCs or their conditioned medium obtained from middle age or elder donors restrained leucocyte infiltration and fibrosis, and resulted in re-epithelialization, dermal-epidermal junction formation and appendage regeneration.
Our data prove that donor age modify the regenerative potential of MSCs and suggest that this might be due to changes in their paracrine factor secretion capability.
Supported by FONDECYT 11110009 to ML.A.

W-3014

TRANSPLANTATION OF HUMAN AMNION-DERIVED MESENCHYMAL STEM CELLS IMPROVES SEVERE COLITIS VIA ATTENUATION OF MACROPHAGE ACTIVITY IN RATS

Shunsuke Ohnishi[1], Reizo Onishi[1], Ryosuke Higashi[1], Kenichi Yamahara[2], Michiko Watari[3], Waka Kobayashi[1], Takehiko Katsurada[1], Hiroshi Takeda[1], Naoya Sakamoto[1]
[1]Hokkaido University, Sapporo, Japan, [2]National Cerebral and Cardiovascular Center, Osaka, Japan, [3]Tenshi Hospital, Sapporo, Japan

Aims: Mesenchymal stem cells (MSCs) have been reported to be a valuable cell source in cell therapy, and bone marrow (BM) represents a major source of MSCs; however, there are several limitations when using BM-MSCs, including inadequate cell numbers and invasiveness. Recently, several studies have shown that MSC can be easily isolated from human amnion, and a large amount of cells can be obtained. We thus examined the therapeutic ef-

fects of transplantation of human amnion-derived MSCs (hAMSCs) in dextran sulfate sodium (DSS)-induced colitis in rats.
Methods: The Medical Ethical Committee approved this examination and all pregnant women gave written informed consent. Amnion was obtained at Cesarean delivery, and hAMSCs were isolated and expanded by digestion with collagenase, followed by culturing in uncoated plastic dishes. Severe colitis was induced in 7-week-old male Sprague-Dawley rats by administration of 8% DSS in drinking water ad libitum from day 0 to day 5. At day 1, hAMSCs (1×106 cells) were transplanted intravenously. Changes in body weight and disease activity index (DAI) were evaluated daily for 5 days. Rats were sacrificed on day 5, and the entire colon was extracted. Colon length, histological colitis score were evaluated, and mRNA expression of inflammatory mediators were measured by quantitative RT-PCR. Infiltration of monocytes/macrophages (CD68), neutrophils (myeloperoxidase (MPO)) and T cells (CD3) was investigated by immunohistochemistry. Effect of hAMSCs on induction of TNF-α from mouse macrophage cell line RAW264.7 by lipoplysaccahride (LPS) was evaluated with quantitative RT-PCR and ELISA.
Results: Transplantation of hAMSCs significantly ameliorated the DAI score, weight loss, colon shortening and histological colitis score. mRNA expression of TNF-α, IL-1β and MIF was significantly decreased in the rectum of hAMSC-treated rats. In addition, infiltration CD68-positive cells was significantly decreased in hAMSC-treated rats. Furthermore, expression of TNF-α mRNA and protein from RAW264.7 cells induced by LPS was significantly attenuated by co-culture with hAM-MSCs, or by culture with conditioned medium obtained from hAMSCs.
Conclusion: Transplantation of hAMSCs provided significant improvement in a rat model of severe colitis, possibly through inhibition of macrophage activity. AMSC would be considered as a new cell source for the treatment of severe colitis.

W-3015

BONE MARROW-DERIVED MESENCHYMAL STEM CELL THERAPY FOR SYSTEMIC ARTERIAL HYPERTENSION IN RATS.

Igor Oliveira Loss, Lucas Felipe Oliveira, Marcos Vinicius Silva, Thalles Ramos Almeida, Marcus Paulo Ribeiro Machado, **Valdo Jose Dias da Silva**
Biological Sciences, Triangulo Mineiro Federal University, Uberaba, Brazil

Previous data from our laboratory have demonstrated that allogenic transplantation of bone marrow mononuclear cells from spontaneously hypertensive rats (SHR) has reduced arterial blood pressure (ABP) around 25-30 mmHg for two consecutive weeks. The central aim of the present work was to examine the therapeutic effects of bone marrow-derived mesenchymal stem cell (MSC) transplantation previously cultivated *in vitro* on spontaneous arterial hypertension in SHR. Adult female SHRs (200 - 230g) were divided in two groups: SHR-MSC (n=8) and SHR-CON (control, n=8). The animals of each group have received respectively an intra-peritoneal injection of 5 millions MSCs (expanded until 5th passage) or vehicle solution (physiological saline). Systolic arterial pressure (SAP) and heart rate (HR) were monitored by means of tail occlusion method 5 days before and after treatment during 15 days. Following, for direct recordings of hemodynamic parameters, all animals were anesthetized with tribromoethanol (250 mg/kg, i.p.) and had their right femoral artery catheterized. After 24-48 hours of surgical recovering, ABP were recorded during 60 minutes, and at the end, after a new anesthesia session (urethane - 1,2g/Kg), a catheter was inserted into left common carotid artery for injection of doses of acetylcholine (3-25ηg/Kg) or sodium nitroprusside (0,5-4μg/Kg), in order to indirectly evaluate systemic endothelial function by means of depressor responses to endothelial-dependent and endothelial-independent vasodilators, respectively. At the end of the experimental protocol, all animals were euthanized and heart, lungs, kidneys, liver, spleen and skeletal muscle (gastrocnemius) were collected, rinsed in physiological saline, fractioned and frozen or fixed in formaldehyde. Heart weight was measured and fixed slides of skeletal muscle were processed for immunohistochemical labeling for CD31 in order to quantify muscle capillary vessels. Fixed slides of kidneys stained with hematoxylin/eosin were used to morphometric studies of renal glomeruli. Pieces of different organs from animals transplanted with MSCs were fractioned, collagenase-digested and cells previously labeled with DiI CM were counted using flow cytometry. Indirect and direct measurements of ABP have shown long lasting reduction (15 days) of tensional levels (around 15-20 mmHg), after i.p. administration of MSCs in SHR. In addition, MSC transplantation has also provoked increas-

ing in spontaneous baroreflex sensitivity and decreasing in ABP variability, as well as reduction in cardiac hypertrophy and glomerular Bowman space in treated animals. Endothelial dysfunction of SHR was not changed by MSC treatment. Searching for DiI CM positive cells in several tissues from transplanted animals have revealed minimal quantities of cells in lungs and kidneys. Taking all together, our findings seem to indicate a promising utilization of bone marrow-derived mesenchymal stem cell transplantation to treat systemic arterial hypertension. Despite the lack of mechanisms, a possible paracrine effect of MSC seems to be possible.

W-3016

MESENCHYMAL PRECURSOR CELLS ATTENUATE THE DEVELOPMENT OF ENDOTHELIAL DYSFUNCTION IN AN OVINE MODEL OF COLLAGEN-INDUCED ARTHRITIS

Laura M. Dooley[1], Elizabeth A. Washington[1], Anwar M. Abdalmula[1], Peter Ghosh[2], Wayne G. Kimpton[1], Silviu Itescu[2], Simon R. Bailey[1]

[1]Faculty of Veterinary Science, The University of Melbourne, Parkville, Australia, [2]Mesoblast Ltd, Melbourne, Australia

Rheumatoid arthritis (RA) is a systemic autoimmune condition associated with increased risk of cardiovascular mortality. The chronic systemic inflammatory response is believed to accelerate the development of subclinical vascular lesions. The earliest detectable cardiovascular change in human RA is dysfunction of endothelial cells, characterised by reduced endothelium-dependent vasodilation. Our earlier studies have demonstrated that an ovine model of rheumatoid arthritis produces systemic endothelial dysfunction. Bone marrow-derived mesenchymal precursor cells (MPC) have been reported to possess immunomodulatory properties that may prove beneficial in autoimmune and chronic inflammatory conditions. The aim of this study was to determine if ovine MPC given early in the disease course of an ovine model of collagen-induced arthritis are able to improve clinical signs and attenuate the development of endothelial dysfunction.

Arthritis was induced in sixteen healthy adult merino sheep by administration of two subcutaneous injections of bovine type II collagen in Freund's adjuvant two weeks apart, followed by an intra-articular injection of bovine type II collagen into the left hock joint. One day following the intra-articular injection, sheep were administered either 150 million allogenic ovine MPCs intravenously (n=8), or saline only (n=8). Sheep were examined frequently to score clinical signs of arthritis. Lameness, swelling and pain on joint flexion were assessed. Plasma samples were collected at days 0, 1, 2, 3, 5, 7, 10 and 13 following treatment to characterise the systemic inflammatory response. Plasma fibrinogen was measured using the modified heat precipitation method at a commercial veterinary laboratory. All animals were culled two weeks following arthritis induction. Coronary arterial segments were mounted in a Mulvaney-Halpern wire myograph, pre-contracted then dilated with the endothelium-dependent dilator bradykinin (10-11M to 3x10-6M). Paired segments were dilated with the endothelium-independent dilator, sodium nitroprusside (SNP, 10-9M to 3x10-4M).

Sheep treated with MPCs demonstrated significantly lowered clinical arthritis scores over the 13 days following treatment ($p<0.05$, unpaired t-test). Coronary arteries from RA sheep treated with MPCs demonstrated a significantly greater maximal relaxation to bradykinin when compared to untreated RA sheep (255.5 ±9.1 % of pre-contracted tone vs. 173.9 ±13.1 % in controls; $p<0.05$, unpaired t-test). There was no significant difference in the response of coronary arteries to SNP between the two groups, indicating that vascular smooth muscle function was unaffected by treatment. Treated sheep also showed significantly reduced circulating levels of the inflammatory marker fibrinogen at days 1,2, 3 and 5 post MPC treatment ($p<0.05$, 2-way ANOVA with Bonferroni post hoc test). This pre-clinical study demonstrated that MPCs given intravenously early in the course of arthritis development are able to attenuate the development of clinical signs, systemic inflammation and endothelial dysfunction in this animal model. The study therefore provides evidence of the efficacy of MPC in modulating the systemic inflammatory effects of collagen-induced arthritis.

W-3018

TEMOZOLOMIDE POTENTIATES ANTITUMOR EFFECT OF MESENCHYMAL STEM CELLS-BASED TRAIL GENE THERAPY AGAINST MALIGNANT GLIOMA

Seong Muk Kim, Ji Sun Woo, Chang Hyun Jeong, Sin-Soo Jeun
The Catholic University of Korea, Seoul, Korea, Republic of

Because the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) selectively kills tumor cells, it is one of the most promising candidates for cancer treatment. TRAIL-secreting human mesenchymal stem cells (MSC-TRAIL) provide targeted and prolonged delivery of TRAIL in glioma therapy. However, acquired resistance to TRAIL of glioma cells is a major problem to be overcome. In this study, we showed a potential therapy that used MSC-TRAIL combined with the chemotherapeutic agent temozolomide (TMZ), which is currently used in clinical practice. Treatment of either TRAIL-sensitive or -resistant human glioma cells with TMZ and MSC-TRAIL resulted in a significant enhancement of apoptosis compared with the administration of each agent alone, indicating that TMZ potentiates TRAIL-induced apoptosis. In addition, we demonstrated that TMZ effectively increased the sensitivity to TRAIL-induced apoptosis via the upregulation of the death receptor 5 and the downregulation of antiapoptotic proteins, such as X-linked inhibitor of apoptosis protein and cellular FLICE-inhibitory protein. Subsequently, this combined treatment resulted in a substantial increase in caspase activation. Furthermore, *in vivo* survival experiments and bioluminescence imaging analyses performed in mice bearing intracranial gliomas showed that treatment using MSC-TRAIL combined with TMZ had greater therapeutic efficacy than did single-agent treatments. Overall, these results suggest that the combination of clinically relevant TMZ and MSC-delivered TRAIL is a potential therapeutic strategy for improving the treatment of malignant gliomas.

W-3021

IMPROVED THERAPEUTIC POTENTIAL OF MESENCHYMAL STEM CELLS VIA MRNA TRANSFECTION

Oren Levy[1], Weian Zhao[1], Luke J. Mortensen[2], Sarah LeBlanc[1], Kyle Tsang[1], Moyu Fu[1], Vinay Sagar[1], Peter Eimon[3], Matthew Angel[3], Charles P. Lin[2], Mehmet Fatih Yanik[3], Jeffrey M. Karp[1]
[1]Division of Biomedical Engineering, Department of Medicine, Center for Regenerative Therapeutics, Harvard Medical School, Harvard Stem Cell Institute, Brigham and Women's hospital, Cambridge, MA, United States, [2]Wellman Center for Photomedicine and Center for Systems Biology, Massachusetts General Hospital, Boston, MA, United States, [3]Department of Electrical Engineering and Computer Science, Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, United States

Cell therapy possesses tremendous potential for treating a wide array of tragic diseases. Specifically, mesenchymal stem cells (MSCs) are considered candidates for cell-based therapy due to their immunomodulatory and pro-angiogenic secretome and low immunogenicity. However, a major challenge in cell therapy, and especially MSC therapy, is poor cell homing to disease sites following systemic administration. In this study, we used mRNA transfection as an approach to engineer MSC homing and immunomodulatory properties to better control their fate following transplantation. Since cell rolling is considered a key step in the process of cell homing, mRNA transfection was used to induce expression of key rolling ligands on the MSC surface, resulting in a robust rolling response on P-selectin-coated surfaces *in-vitro* as well as on inflamed endothelium *in-vivo*. Moreover, the engineered MSCs exhibited significantly increased homing to sites of inflammation following systemic administration in murine *in-vivo* models. We then generated MSCs that express a functional rolling machinery and a potent anti-inflammatory cytokine. Systemic infusion of MSCs equipped with both homing ligands and an enhanced immunomodulatory secretome showed the greatest local anti-inflammatory effect *in-vivo*. Overall, this study utilizes mRNA transfection to implement a combined approach - enhancing MSC homing as well as augmenting its secretome – to improve MSC therapeutic potential.

W-3022

TRANSPLANTATION OF HYALURONIC ACID COATED WHARTON'S JELLY MESENCHYMAL STEM CELLS CAN REPAIR LIVER FIBROSIS

Chang-Chun Hsiao[1], Fang-Cheng Huang[2], Te-Yao Hsu[3], TSUNG-CHIH HSIEH[2], HSIN-I CHEN[1]

[1]Graduate Institute of Clinical Medical Sciences, Chang Gung University, Kaohsiung, Taiwan, [2]Department of Chemical and Materials Engineering, Cheng Shiu University, Kaohsiung, Taiwan, [3]Department of Obstetrics, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Kaohsiung, Taiwan

Liver fibrosis (LF) is mainly due to viral hepatitis, autoimmune diseases, alcoholism or chemical drug-induced liver damage caused by a long time. Mesenchymal stem cells (MSC) with immunosuppressive and anti-inflammatory functions, the use of MSC to treat LF are a very feasible. Using of hyaluronic acid (HA)-coated Wharton's jelly MSC (WJMSC) can reduce WJMSC apoptosis and increase proliferation of WJMSC. We use carbon tetrachloride to establish stable and reliable animal model of LF. We use HA-coated 6×10^6 WJMSC transplanted into the liver capsule of LF mice (HA-coated WJMSC treated mice). After 28 days of treatment, type I collagen expression in the liver tissue of HA-coated WJMSC treated mice recovery to same level as normal mice, there is no biological marker of activation of hepatic stellate cells α-SMA expression. Albumin in liver tissue of HA-coated WJMSC treated mice increased significantly to about that 1.9 ± 0.1 fold ($p < 0.01$) of the normal mouse liver tissue. Transplantation HA-coated WJMSC reduced LF mice GOT, GPT, and liver fibrosis area. After 28 days of treatment, in HA-coated WJMSC treated mice liver tissue, there are still a lot of WJMSC and MMP9 expression is very large. MMP9 has been reported in the literature can degrade collagen fibers. Both IL-6 and TGF-β expression in the liver tissue of HA-coated WJMSC treated mice are significantly decreased ($p < 0.05$) compared to those of LF mice and restored to those of normal mice. Therefore, transplantation of HA-coated WJMSC is indeed effective in treatment of LF mice to liver function recovery and reduce the degree of liver fibrosis.

W-3023

NEW INSIGHTS INTO THE IMMUNOMODULATORY ACTIVITY OF MESENCHYMAL STEM CELLS: IMPLICATIONS FOR TRANSLATIONAL AND CLINICAL RESEARCH

Micheline M. Resende[1], Maria Graca Cabreira-Hansen[1], Xiaohong Wang[1], Aaron Orozco[2], Sarah Schneider[2], Christina Bove[1], James JT Willerson[3], Doris Taylor[2], Emerson Perin[1]

[A]Stem Cell Center, [B]Regenerative Medicine, [1]Texas Heart Institute, Houston, TX, United States

Mesenchymal stem cells (MSC) are reported to have a potent immunosuppressive capacity that prevents tissue damage and increases regenerative potential after injury, a property that has been touted as beneficial in a variety of diseases. Even though the swine model has been used in pre-clinical studies to evaluate the safety and efficacy of MSC therapy, the immunomodulating properties of swine-derived MSCs has not been completely defined. Contradictory results have been reported by several research groups using porcine MSCs from different strains. Strain-specific differences in MSC immunomodulating properties may account for these discrepancies and could have implications for translational studies. Objective: The goal of this study is to compare immunomodulatory activity of human MSCs to two different strains of swine MSCs (Yucatan and Yorkshire) under control and cytokine activated conditions in vitro. Methods: MSCs were isolated from 9 healthy humans, 6 Yucatan and 5 Yorkshire pigs. MSCs were stimulated with interferon-γ (IFN-γ) for 24 h and the levels of major histocompatibility complex class II (MHC-II) expression and anti-inflammatory cytokines: interleukin 10 (IL-10), and transforming growth factor beta (TGFβ) evaluated by flow cytometry and ELISA, respectively. Inhibition of phytohemagglutinin (PHA)-induced T-cell proliferative responses were also quantified using MSC co-cultures. Results: No baseline differences were observed in MHC-II expression between human (0.06%) and porcine (Yucatan=1.1 %; Yorkshire= 0.4%) untreated MSCs. However, IFN-γ treatment induced a significant increase in MHC-II expression in porcine MSCs when compared to baseline and this increase was significantly higher in Yorkshire compared to Yucatan MSCs (Yorkshire:32.6% vs. Yucatan 8.38%,P=0.03). Human MSCs released significantly higher levels of TGFβ(1.7-fold, p=0.01) after IFN-γ treatment that was not observed in porcine MSCs (Yucatan 0.97 fold, Yorkshire 0.77 fold), indicating that human MSCs may

have greater immunomodulatory activities since TGFβ has potent anti-proliferative and anti-inflammatory activities. In fact, allogeneic human MSCs significantly suppressed T cell proliferation in a dose-dependent manner. This effect was independent of MSC direct contact with T-cells, since T-cell proliferation was also suppressed in a transwell assay. In contrast, pig MSCs (Yorkshire or Yucatan) were not able to suppress T-lymphocyte proliferation in vitro. Conclusion: In conclusion, this study demonstrates that regulation of MHC-II differs by porcine strain after IFN-y stimulation, and that at least two commonly used pig strains differ from human in their immunomodulatory response. These in vitro immunomodulatory differences between human and pig MSCs raise the question of pig as a predictive animal model especially in immune-mediated diseases, suggesting direct in vivo comparisons are necessary.

W-3024

MICRORNA-21 ENHANCES VEGF SECRETION OF HUMAN SYNOVIAL FLUID DERIVED MESENCHYMAL STEM CELLS BY TARGETING PTEN

Chang Youn Lee[1], Byeong-Wook Song[2], Onju Ham[2], Se-Yeon Lee[2], Jun-Hee Park[1], Jiyun Lee[2], Hyang-Hee Seo[2], Minji Seung[2], Eunhyun Choi[3], Eunmi Choi[4], Il-Kwon Kim[6], **Ki-Chul Hwang**[5], Woochul Chang[7]
[1]Graduate School, Yonsei University, Department of Integrated Omics for Biomedical Sciences, Seoul, Korea, Republic of, [2]Yonsei University College of Medicine, Brain Korea 21 Project for Medical Science, Seoul, Korea, Republic of, [3]Yonsei University Health System, Severance Integrative Research Institute for Cerebral & Cardiovascular Disease,, Seoul, Korea, Republic of, [A]Yonsei University College of Medicine, [4]Severance Biomedical Science Institute, Seoul, Korea, Republic of, [5]Cardiovascular Research Institute, Seoul, Korea, Republic of, [6]The Catholic University of Korea College of Medicine, Institute of Catholic Integrative Medicine, Seoul, Korea, Republic of

Although many studies show the possibility of stem cell therapy for the regeneration of myocardial infarction (MI), the poor viability and the functional disruption of stem cells in pathologic conditions critically have an influence on the efficacy of stem cell-based therapy. Synovial fluid-derived mesenchymal stem cells (SF-MSCs) are one of the newly reported mesenchymal stem cells and it is expected to be a promising new source of stem cell-based therapy. MicroRNAs (miRs) are short non-coding RNA and play important roles in various biological functions including differentiation, proliferation, and apoptosis by negatively regulating post-translation. In particular, miR-21 has been reported that the effects on cell survival and angiogenesis. In this study, we transfected hsa-miR-21 mimic into human SF-MSCs. Overexpression of miR-21 increased cell survival and angiogenesis-related factors such as phosphorylated AKT and VEGF by targeting phosphatase and tensin homolog (PTEN). We found that luciferase activity and protein expression were diminished in miR-21-transfected SF-MSCs by using luciferase reporter construct containing the 3' UTR of PTEN, as a possible target of miR-21. Using rat MI model, we detected the improvement of cardiac function in miR-21-transfected SF-MSC transplanted group *in vivo*. Our results suggest that miR-21 targeting PTEN contributes to the regeneration of injured heart by transplantation of SF-MSC.

W-3025

MICRORNA-124 SIMULTANEOUSLY TARGETS PDCD6 AND DAPK1 FOR CELL SURVIVAL IN HYPOXIC RAT MESENCHYMAL STEM CELLS

Onju Ham[1], Byeong-Wook Song[1], Min-Ji Cha[1], Se-Yeon Lee[1], Chang Youn Lee[2], Jun-Hee Park[2], Jiyun Lee[1], Hyang-Hee Seo[1], Minji Seung[1], Eunhyun Choi[3], Eunmi Choi[4], Il-Kwon Kim[5], **Ki-Chul Hwang**[4]
[1]Yonsei University College of Medicine, Brain Korea 21 Project for Medical Science, Seoul, Korea, Republic of, [2]Graduate School, Yonsei University, Department of Integrated Omics for Biomedical Sciences, Seoul, Korea, Republic of, [3]Yonsei University Health System, Severance Integrative Research Institute for Cerebral & Cardiovascular Disease, Seoul, Korea, Republic of, [4]Yonsei University College of Medicine, Severance Biomedical Science Institute,

Seoul, Korea, Republic of, [5]Yonsei University College of Medicine, Cardiovascular Research Institute, Seoul, Korea, Republic of

Although mesenchymal stem cells (MSCs) are suitable source for regenerative therapy, the poor survival of MSCs under harsh condition limits the efficacy of cell therapy. MicroRNAs (miRNAs), short non-coding 18~25 nucleotide RNAs, regulate various pathophysiological functions by negatively regulating gene expression. However, the exact mechanism that specific miRNAs could regulate cell survival is still not fully understood. Here, we founded that programmed cell death 6 (PDCD6) and death-associated protein kinase 1 (DAPk1), i.e. pro-apoptotic proteins, are specific targets of miR-124 in hypoxic rat MSCs. We validated the suppression of PDCD6 and DAPk1 in miR-124-transfected cells. Overexpression of miR-124 increased MSC survival in hypoxic condition and specifically regulated the apoptosis and survival-related proteins including phosphorylated-Akt and -ERK1/2, Bcl-2, Bax, cytochrome c, and caspase-3. In addition, annexin V and PI-stained cells were decreased in miR-124-up-regulated group. MSC survival in hypoxic condition was enhanced in miR-124-treated group compared to PDCD6- or DAPk1-siRNA-treated group, respectively. These results represent that miR-124 simultaneously targets PDCD6 and DAPk1 for enhancement of survival in hypoxic MSCs.

W-3026

INTRA-ARTERIAL DELIVERY OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS (ADSC) LEADS TO FUNCTIONAL IMPROVEMENT IN A RODENT MODEL OF MIDDLE CEREBRAL ARTERY OCCLUSION

Chunggab Choi[1], Seung-Hun Oh[1], Da-Jeong Chang[1], Nayeon Lee[1], Iksoo Jeon[1], Seyoung Hwang[1], Hyun Sook Kim[1], Ki-Baik Hahm[2], Young Seok Park[1], Dong Ah Shin[3], Hyunseung Lee[4], Kwan Soo Hong[4], **Jihwan Song**[1]
[A]CHA Stem Cell Institute, Department of Biomedical Science, [B]Department of Gastroenterology, [1]CHA University, Seoul, Korea, Republic of, [2]Department of Neurosurgery, Yonsei University College of Medicine, Seoul, Korea, Republic of, [3]Division of Magnetic Resonance, Korea Basic Science Institute, Ochang, Korea, Republic of

Stroke is one of the major causes of death and disability. It is mainly caused by the blockade of blood flow, leading to the ischemic condition that causes irreversible damages to the brain. Despite numerous efforts, therapeutic options for the stroke-damaged patients are still very limited. In this study, we evaluated the therapeutic potential of adipose-derived mesenchymal stem cells (ADSC) to treat stroke using a rodent model of middle cerebral artery occlusion. ADSC are known to secrete many useful cytokines or growth factors, such as BDNF, VEGF, GDNF, HGF, which will facilitate the protection and recovery of stroke-damaged tissues (MCAo). For the delivery route of stem cells, we chose intra-arterial infusion method, since it results in the higher cell counts at the target site, compared with intravenous infusion method. However, since this method can also create the potential risk of embolization of the injected vessels, etc., we carefully monitored the progression of transplantation effects or the occurrence of post-operational side effects. ADSC were infused through internal carotid artery at 24hr after the induction of MCAo, and the transplanted animals were monitored up to 8 weeks. Interestingly, we observed that the transplanted animals exhibited significant improvement in motor and somatosensory functions, judged by rotarod and mNSS (modified neurological severity score) tests, respectively. 4.7T animal MRI analysis indicated that Feridex-labeled ADSC were mainly detected in the infarct area, implying the tropism of infused stem cells. Unlike the previous reports, we were able to detect considerable numbers of transplanted ADSC even 8 weeks after infusion. Immunohistochemical analyses revealed that small portions of transplanted ADSC were differentiated into both NeuN- or MAP2-positive neuronal or GFAP-positive glial lineages. Interestingly, some of the transplanted cells were also differentiated into laminin- or vWF-positive endothelial cell lineages, suggesting that the transplanted ADSC might be also involved in angiogenesis. We also observed that the transplanted animals exhibited increased neurogenesis (higher BrdU and DCX expression), and reduced inflammation (lower Iba-1 and ED1 expression) and glial scar formation (lower GFAP expression). Taken together, these results indicate that, although ADSC cannot give rise to dramatic functional recovery and neuronal regeneration *per se*, wide spectrum of paracrine effects of ADSC can improve the host environment by modulation of neurogenesis, angiogenesis, inflammation, gliosis, etc. It is likely that these unique features of ADSC will collectively contribute to functional improvement in stroke.

This study was supported by a grant of the Korea Healthcare technology R&D project, Ministry for Health,Welfare & Family Affairs (A111016). We are grateful to the MRI facility in the Division of Magnetic Resonance, Korea Basic Science Institute, Ochang, Korea

W-3027

A POTENTIAL DELIVERY VEHICLE FOR THERAPEUTIC GENES OF SYNOVIAL FLUID DERIVED HUMAN MESENCHYMAL STEM CELLS FROM OSTEOARTHRITIS PATIENTS IN GLIOMA

Sang In Park
Institute of Catholic Integrative Medicine, Incheon St.Mary's Hospital, The Catholic University of Korea College of Medicine, Incheon, Korea, Republic of

A potential delivery vehicle for therapeutic genes of synovial fluid derived human mesenchymal stem cells from osteoarthritis patients in glioma

Sang In Park[a], Yoonjin Bae[a], Hyun-joo Ham[a], Yu Jin Jung[a], Moon-Seo Park[a], Yong-An Chung[a], Lee-So Maeng[a], Woo-chul Chang[a]

[a]Insitute of catholic Integrative Medicine (ICIM), Incheon St. Mary's Hospital, The Catholic University of Korea.

Abstract

A number of studies have demonstrated the tropism of mesenchymal stem cells (MSCs) in malignant gliomas, making these cells a potential vehicle for delivery of therapeutic gene to disseminated glioma cells. The synovial fluid-derived MSCs (SFMSCs) from osteoarthritis patients are attractive resource for potential clinical application. In this study, we compared the tumor-tropic migratory capacities of various MSCs such as bone marrow-derived MSCs (BMMSCs), adipose tissue-derived MSCs (ADMSCs), and SFMSCs. We confirmed the migratory capacity of MSCs toward tumor cells by an *in vitro* migration and by *in vivo* injection of NIR-675-labeled MSCs into the tumor mass of established human luc-U87-glioma in nude mice. Also, we examined whether soluble factors released by glioma cells could change migration and adhesion related factors and microRNAs (miR) of MSCs. All the MSCs showed a significantly increase migratory capacity toward glioma *in vitro* and *in vivo*. Moreover, SFMSCs showed enhanced the migration capacity toward glioma compared to ADMSCs. Also MSCs treated of conditioned medium increased expression of CD44 and CD49a, which lead to the change of the CD44 targeted- miR-33a-3p and CD49a targeted-miR-124a-5p on MSCs. These results suggest that SFMSCs may have potential use as effective delivery vehicles for therapeutic genes in the treatment of glioma.

W-3028

CHARACTERIZATION OF THE DYNAMIC MOLECULAR CHANGES IN STEM CELL CHEMOATTRACTANTS FOLLOWING PULSE FOCUSED ULTRASOUND (PFUS) SONICATIONS IN MUSCLE: IMPLICATIONS FOR CELL THERAPY

Pamela A. Tebebi[1], Scott R. Burks[2], Ben A. Nguyen[2], Saejeong J. Kim[2], Ali Ziadloo[2], Priyanka Venketesh[2], L. Christine Turtzo[2], Victor Frenkel[1], Joseph A. Frank[2]

[1]Biomedical Engineering, The Catholic University of America, Washington, DC, United States, [2]Radiology and Imaging Sciences, National Institutes of Health, Bethesda, MD, United States

Introduction: Pulsed focused ultrasound (pFUS) has been used as a noninvasive nondestructive technique to enhance tissue permeability and retention in targeted drug delivery. We previously reported elevations in cytokines, chemokines and trophic factors (CCTF) associated with minimal inflammatory changes in the targeted muscle following pFUS, and that pre-treating skeletal muscle or kidney tissue with pFUS induces an enhanced homing, permeability, and retention (EHPR) effect for intravenously-injected mesenchymal stem cells (MSC) in skeletal muscle and kidney tissue. The goal of the present study was to characterize changes in these factors over time in the murine muscle in response to pFUS. Results may point to important signaling pathways that drive the molecular changes following pFUS and reveal an optimum time to administer cells to maximize the EHPR effect and ultimately translate into improved cellular therapies.

Methods: Hamstrings of C3H mice were sonicated using a Sonoblate 500 system at 1 MHz. Sonications were done at 40 W (5% duty cycle, 1 Hz pulse frequency). 100 pulses per site were given in a 2 × 3 matrix (spacing = 2 mm). Treated hamstrings were harvested at various times up to 48 hr post treatment with sham-treated mice representing controls. Tissue homogenates were analyzed by ELISA for levels of CCTF's, and cell adhesion molecules (CAM) at each time point (n=6 per time point). 1×106 human MSC were injected intravenously within 2 hr following pFUS treatment. For ibuprofen experiments, mice were given 30 mg/kg ibuprofen by mouth 15 min prior to pFUS.
Results & Discussion: A biphasic response to pFUS occurs as an initial increase in CCTF (30min-2hr) followed by a second peak of chemoattractive factors at 16 hr that decays through 48hrs. The overall trend was consistent with previously published studies where the early elevation of chemoattactants (i.e., IL17, IL12, M-CSF, VEGF) following pFUS likely drives the expression of other CCTF detected at later time points. Accompanying increased expression of soluble factors was an upregulation CAMs. When MSC are injected <4 hr post-pFUS, homing to the treated region was greatest. However, when injections were performed at 8 hr or 16 hr post-pFUS the magnitude of cell homing was reduced. Additionally, when mice are given ibuprofen prior to pFUS and cell injections, MSC homing to treated tissue does not occur.
Conclusions: The major finding is that the initial mechanical perturbation of the muscle by pFUS results in dynamic molecular biological effects that have important implications on the ability to maximize the EHPR effect for cell therapies. The timecourse of CCTF changes after pFUS indicate that the predominantly mechanical (i.e. non-thermal) effects, through mechanotransduction, give rise into an initial molecular biological response that is responsible for the second peak that occurs at the later time points. While this cascade of molecular changes can capitalized upon to increase homing of MSC, the administration of ibuprofen, a non-steroidal anti-inflammatory drug, blocks homing to pFUS-treated tissue. This suggests that pFUS effects that lead to MSC homing could be mediated by cyclooxygenase (COX) activity. Understanding and exploiting CCTF changes following pFUS provide a mechanism to appropriately time infusion of cell products, screen drug effects on cell homing, and improve delivery of cell therapies for diseases and regenerative medicine.

W-3031

CELL THERAPY WITH MESENCHYMAL STEM CELLS IN RENAL DAMAGE INDUCED BY IONIZING RADIATION IN AN ANIMAL MODEL.

Vitoria M. Longo, Helena R.C. Segreto, Waldemar S. Almeida, Nestor Schor
Nephrology Division-Medicine Dept, Escola Paulista de Medicina/UNIFESP, Sao Paulo, Brazil

INTRODUCTION AND AIMS: Ionizing radiation (IR) causes cells death depending on the total irradiated dose and upon biological factors in the specific organ. The IR on the kidney's tissue induces time-depending systemic effects (hypertension, proteinuria, anemia, platelet aggregation, and others) as well as renal alterations (reduction glomerular function and diffuse sclerosis etc.). The reparative therapy with mesenchymal stem cells (MSCs) potentially can minimize renal injury alterations induced by IR. AIM: To evaluate the effect of therapy with MSCs in the renal lesions induced by IR. METHODS: MSCs were obtained from male Wistar rats and characterized by FACS. We formed four groups of 9 animals each: control (CTL); vehicle (V); ionizing radiation (IR) in the right kidney, with a dose of 8Gy and, group treated with IR and cell therapy (1X106cells, IR+MSCs). The rats were placed in metabolic cages for analysis of creatinine (CreatS, mg/dL), creatinine clearance (Clcreat, ml/min) and proteinuria (mg/24h) in the 20th and 40th days post-IR. We used immunofluorescence for Y chromosomal location and immunohistochemistry (IHC) for marking inflammation and fibrosis, in both periods.
RESULTS: In day 20 when comparing groups IR and CTL, we found increase in mean Creat (0.96±0.19 vs. 0.34±0.02) and reduction in Clcreat (0.36±0.14 vs. 1.19±0.08), $p<0.05$. Unlike occurred in relation to the IR group alone, animals that received MSCs (group IR+MSCs) presented lower CreatS (0.96±0.19 vs. 0.42±0.04) and higher Clcreat (0.36±0.14 vs. 0.79±0.12), $p<0.05$. In 40th day, the IR group showed an additional aggravation on renal function with significant increase in proteinuria when compared to the CTL and IR+MSCs (31.3±5.07 vs. 10.8±0.08 and 13.4±1.69; $p<0.05$), indicating that the renal function loss process is continuous and could blocked by MSCs transplantation. At 40th day of the evaluation there was a progressive impairments in the levels of CreatS (1.36±0.04 vs. 0.41±0.04 and 0.39±0.03) and Clcreat (0.47±0.08 vs. 1.01±0.13 and 1.06±0.20) in IR group, when compared to

IR+MSCs and CTL; p<0.05, indicating that the protective effects of MSCs, in this model, had a long term efficiency. Elevated levels of inflammatory marker for IL-2 and IL-6 were found in IR but blunted with MSCs treatment. The Y chromosome was persistent in IR+MSCs, indicating a continuous process of reparation.
CONCLUSIONS: Maintenance in Creats and Clcreat levels given by MSCs, in both periods were achieve. The Clcreat and proteinuria are normal after the 40th day in the RI+MSCs. The MSCs prevented the aggressive effect on renal function at 20 days post-IR, and attained normalcy after the 40th day. IHC showed that the presence of a chronic inflammatory process participate, as expected, in the development of this disease. The markup for IL-6 was absent in group IR+MSCs vs. IR, suggesting that MSCs prevented the aggressive effect of acute inflammation. The presence maintained of Y chromosome in IR+MSCs indicated persistence of the harmful inflammatory process despite no more IR aggression. Finally, the data showed that treatment with MSCs induced beneficial effects on the renal damage provoked by IR, and the MSCs transplantation induced beneficial effects in this experimental model.
Supported by FAPESP, CNPq, CAPES and FOR

W-3032

EFFECTS OF MESENCHYMAL STEM CELLS OR THEIR CONDITIONED MEDIUM IN UNILATERAL URETERAL OBSTRUCTION IN RATS

Andrei Furlan da Silva, Vicente Paulo Teixeira, Nestor Schor
Nephrology Division-Medicine Dept, Escola Paulista de Medicina/UNIFESP, Sao Paulo, Brazil

Introduction and objectives: A substantial knowledge has been accumulated in relation to therapeutic potential of mesenchymal stem cells (MSCs) and its conditioned medium (CM). It is known their ability to repair tissue and reduce local inflammation. A renal tubule interstitial inflammation is one of the principal causes that induce chronic damage, resulting in fibrosis. A model well established for fibrosis is unilateral ureteral obstruction (UUO). In this study we evaluated the effects of MSCs or CM administrations in UUO model. Methods and results: MSCs extracted from rat's bone marrow were cultivated in vitro and characterized by flow cytometry and cellular differentiation. Four groups of female rats were employed in vivo (n=7): SHAM; UUO; UUO+ MSCs and UUO+CM. The MSCs or its CM were administered via cava vein after total left ureter ligation. After 7 days, the rats were sacrificed and serum and UUO kidney were collected. The fibrosis was assessed by deposition of collagen type I and III displayed on staining Picro Sirius Red. Results: We observed a significant improvement in reduction of the fibrosis progression in animals treated with MSCs or CM (UUO 1.2±0.2; MSC 0.3±0.1; CM 0.4±0.1, % of staining area, p<0.05). The expression of molecules related to progression of fibrosis, inflammation and Epithelial-Mesenchymal Transition were evaluated by real time PCR. It was observed reduction in molecules expression such as COL-1(UUO 4.5±0.5; MSC 2.3±0.3; MC 1±0.2, arbitraries unities), α-SMA (UUO 5.2±0.5; MSC 3±0.5; MC 2.7±0.3, arbitraries unities) and TNF-α (UUO 7.7±1.3; MSC 2.0±0.3; MC 1.7±0.1, arbitraries unities) in animals treated with MSCs or MC, p<0.05. In immunohistochemical assays they were observed reductions of 1,5 % in staining area of capase-3 and α-SMA (UUO 7.2±1; MSC 1.0±0.2; CM 0.7±0.1, % of staining area, p<0.05) in treated animals. We also observed a reduction in cell proliferation after MSCs or MC (UUO 3.1±0.7; MSC 0.4±0.1; CM 0.6±0.3, % of staining area, p<0.05). Conclusion: Results suggested that the IV administration of MSCs or its CM minimize the fibrosis progression and change factors involved in apoptosis, inflammation, cell proliferation and Epithelial-Mesenchymal Transition in Wistar rats, subjected to unilateral ureteral obstruction.
Supported by: FAPESP, CNPq, CAPES and FOR

W-3033

AUTOLOGOUS BONE MARROW STEM CELL TRANSPLANTATION IN PATIENTS WITH CHRONIC SPINAL CORD INJURY

Milena Soares[1], BRUNO SOUZA[1], MARCUS VINICIUS MENDONÇA[3], Ticiana Larocca[4], CRISTIANE FLORA VILLAREAL[8], MARCO ANTONIO NOVAES[5], ANDRÉ COSTA MATOS[6], LUIZ FLÁVIO MAIA SILVA[2], SISSI BRANDÃO FURTADO[7], Carla Martins Kaneto[4], GERALDO PEDRAL SAMPAIO[4], RICARDO RIBEIRO DOS SANTOS[4]

[A]Centro de Pesquisas Gonçalo Moniz, [1]Fundação Oswaldo Cruz, Salvador, Brazil, [2]Neurocirurgia, Hospital Espanhol, Salvador, Brazil, [A]Centro de Biotecnologia e Terapia Celular, [3]Hospital São Rafael, Salvador, Brazil, [C]Urologia, [D]Centro de Pesquisas Gonçalo Moniz, [3]Hospital São Rafael, Salvador, Brazil, [4]Universidade Federal da Bahia, Salvador, Brazil

Introduction and objectives: There is currently no therapy capable of improving neurological functions in paraplegic patients with chronic complete spinal cord injury (SCI). The administration of bone marrow mesenchymal stem cells (MSC) is a potential therapy for different diseases, including those affecting the spinal cord. Here we evaluated the feasibility, safety and potential efficacy of the transplantation of autologous MSC transplantation in patients with chronic complete SCI. Methods and results: This is an open label phase I/II study, non-controlled, which included 14 patients, aged between 18-65 years, with chronic traumatic SCI (> 6 months before the beginning of the trial), classified as ASIA A, without complete anatomical section of the spinal cord, at thoracic or lumbar segments. All of the patients selected for this trial had previously undergone spinal cord decompression and stabilization. Baseline somatosensory evoked potentials (SSEP), magnetic resonance imaging (MRI) of the spine, and urodynamic study were assessed before isolation of MSCs, and in different time points during a minimum 6-months follow-up. Bone marrow was aspirated from the anterior and posterior iliac crests and MSCs were isolated and cultured in a cGMP facility for approximately 4 weeks, until adequate numbers for transplantation were achieved. Before transplantation, the cells were characterized by flow cytometry analysis, differentiation assays, and G-band karyotyping. Patients underwent laminectomy and durotomy, when the cells were injected directly into the injured area of the spinal cord, in cardinal points. A fixed number of cells per volume of lesion (5x106 cells/cm3) was injected, as determined by MRI analysis. No adverse events of the technique were observed, except for one patient who underwent a second surgical procedure due to a liquoric fistula. The evaluation of clinical parameters showed improvements related to sphincter control and sensitivity in lower limbs. None of the patients evolved with worsening of the urinary function and two patients evolved with normalization of the maximum cystometric bladder capacity. Ten patients had improvement of tactile sensitivity and six patients had motor improvement, as quantified by the ASIA score. Fifty percent of the patients evolved with improved ASIA grades to B or C. One patient presented SSEP potential three months after cell transplantation. Conclusions: Transplantation of autologous BMMC into the lesion of patients with spinal cord injury is safe and feasible. Our results also indicate some benefits of the technique, which should be confirmed in further studies.

W-3034

EARLY MARKERS OF CELLULAR SENESCENCE IN BONE MARROW-DERIVED MESENCHYMAL PROGENITOR CELLS

Maria da Graca Cabreira, Micheline M. Resende, Xiaohong Wang, Guilherme V. Silva, Daniel Garcia, Doris A. Taylor, Emerson C. Perin
Stem Cell Center, Texas Heart Institute, Houston, TX, United States

Cellular senescence, an innate part of the organismal aging process, can also be induced by insults generated from diseases. Studies have shown that senescent cells disable the proliferation machinery but remain metabolically active and, if not cleared by the immune system, may pose risks for tumor development. We hypothesized that patients suffering from heart failure, a chronic cardiovascular disease, that might inflict systemic cell damage, could be affected by an increase in cellular senescence.
We collected tissue discard from anonymous heart failure patients undergoing bone marrow aspiration (IRB HSC-MS-09-0527, University of Texas Committee for the Protection of Human Subjects; N=4) for the isolation of mesenchymal/stromal progenitor cells (MPC). Control MPC were isolated from healthy marrows (N=4) purchased from Lonza, Inc. Cells were expanded in growth medium containing 10% fetal bovine serum at 500/cm2 plating density in 5 and 20% O2 tension. Cell characteristics were fully quantified by immunophenotype, clonogenic activity, analysis of growth kinetics and differentiation potential. Transcriptome analysis by microarray was performed on control MPC grown in 5% and 20%. The abundance of 86 senescence-associated transcripts in control and patient cell cultures was compared by PCR array (SABiosciences). Analyses were performed on cells at log phase in the third passage. In terms of cell characteristics, we found no differences between control and patient samples. We found

that MSC from patients when grown at 5% O2 expressed significantly lower levels of Chek1 (2.8 fold; p= 0.007), Id1 (3.5 fold; p=0.01) and PCNA (2.5 fold; p=0.01). We compared expression profiles of cultures expanded in 5% O2 tension to samples in 20% so we could also evaluate whether or not the changes on the senescence profile could be attributed to possible stress caused by culture conditions. 5% O2 tension proved to be the most adequate for the analysis because the results were in agreement with in vivo and in vitro published data. In addition, higher than control protein expression levels of p16, an important senescence marker not included in the array, was detected in patient MPC grown under 5% O2 tension by Western blotting, in agreement with the decreased levels of Id1.
Our screening for senescence markers was performed at early population doublings, when cells retained a relatively robust proliferation capacity because our goal was to obtain a consistent pre-replicative senescence gene expression profile or signature from cultured cells that represents the intrinsic aging occurring in the patient sample that could also reveal pathophysiological processes involved in it. These findings are very preliminary and will need to be validated in larger cardiovascular patient population. We are presently investigating the involvement of Smurf2 on ID1 ubiquitination and upregulation of p16 in patients' samples.

W-3035

THE SCTIVATION OF DIRECTIONAL STEM CELL MOTILITY BY GREEN LIGHT-EMITTING DIODE IRRADIATION

Oscar Lee[1], **Jennifer Ho**[2]
[1]Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan, [2]Center for Stem Cell Research, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan

Light emitting diode (LED) irradiation is potentially a photostimulator to manipulate cell behavior by opsin-triggered phototransduction and thermal energy supply in living cells. Directional stem cell motility is critical for the efficiency and specificity of stem cells in tissue repair. We explored that green LED (530 nm) irradiation directed the human orbital fat stem cells (OFSCs) to migrate away from the LED light source through activation of extracellular signal-regulated kinases (ERK)/MAP kinase/p38 signaling pathway. ERK inhibitor selectively abrogated light-driven OFSC migration. Phosphorylation of these kinases as well as green LED irradiation-induced cell migration was facilitated by increasing adenosine triphosphate (ATP) production in OFSCs after green LED exposure, and which was thermal stress-independent mechanism. OFSCs, which are multi-potent mesenchymal stem cells isolated from human orbital fat tissue, constitutionally express three opsins, i.e. retinal pigment epithelium-derived rhodopsin homolog (RRH), encephalopsin (OPN3) and short-wave-sensitive opsin 1 (OPN1SW). However, only two non-visual opsins, i.e. RRH and OPN3, served as photoreceptors response to green LED irradiation-induced OFSC migration. In conclusion, stem cells are sensitive to green LED irradiation-induced directional cell migration through activation of ERK signaling pathway via a wavelength-dependent phototransduction.

W-3036

CHARACTERIZING THE IMMUNOSUPPRESSIVE ACTIVITY OF HUMAN MULTIPOTENT STROMAL CELLS USING A MOUSE MODEL OF AUTOIMMUNE DISEASE

Cristina Nazarov, Jessica LoSurdo, Steven Bauer, Cheng-Hong Wei
CBER, FDA, Bethesda, MD, United States

Multipotent stromal cells (MSCs) are known to have immunosuppressive activity. They are postulated to evade allospecific T cell responses, making them promising tools for reducing the severity of graft vs. host disease and other autoimmune diseases. Although the immunosuppressive effect has been extensively studied *in vitro*, the molecular mechanisms *in vivo* remain elusive.

In our *in vivo* model, we first activate $CD8^+$ T cells specific for a pancreatic islet antigen, then we inject them (i.v.) in NOD/Scid mice to induce type 1 diabetes (T1D). One day prior to the $CD8^+$ T cell transfer, we infuse the NOD/Scid mice with human MSCs (hMSCs) (i.p.) and monitor blood glucose levels every 3 days.

In this study we show that we were able to induce T1D within 3 weeks after injection of antigen-specific autoreactive $CD8^+$ T cells into NOD/Scid mice. Injection of hMSCs prior to the $CD8^+$ T cells delayed the onset of disease by approximately 2 months when compared with mice not injected with hMSCs. A second injection of $CD8^+$ T cells in euglycemic, hMSCs treated mice resulted in rapid disease progression, suggesting that the hMSCs only temporarily retained in NOD/Scid mice. Injection of hMSCs after the $CD8^+$ T cells did not protect mice from developing the disease, suggesting a role for the microenvironment in priming the hMSCs for immunosuppression. Moreover, the immunosuppressive effects seem to be specific to MSCs; transfer of human fibroblasts instead of hMSCs showed no protection from T1D in our model.
Further studies are underway to investigate the mechanisms underlying the immunosuppressive function of hMSCs *in vivo* and to distinguish between the immunosuppressive capacities of hMSCs from different donors and culture passages.

W-3037

HOMING OF TOPICALLY APPLIED MESENCHYMAL STEM CELLS TO THE BRAIN PARENCHYMA IN AN EXPERIMENTAL TRAUMATIC BRAIN INJURY MODEL

PK Lam[1], Anthony WI Ip[2], Kevin KW Wang[4], Henry CH Lau[1], Kevin KC Leung[3], Kathy TC Li[3], Paul BS Lai[3], WS Poon[3]
[A]Dept of Surgery, Chow Tai Fook-Cheng Yu Tung Surgical Stem Cell Research Center, [B]Dept of Anatomical & Cellular Pathology, [C]Dept of Surgery, [1]The Chinese University of Hong Kong, Hong Kong, Hong Kong, [2]Center of Neuroproteomic & Biomarkers, University of Floria, Florida, FL, United States

Mesenchymal stem cells (MSCs) have the capabilities of neuroprotection and promoting functional recovery in traumatic brain injury (TBI). To maximize the biologic effects, MSCs should be delivered to the target region. In most clinical settings, MSCs are commonly transplanted by systemic infusion. Due to the blood-brain barrier and pulmonary trapping, engraftment rate of the infused MSC into the brain parenchyma is very low. Direct implantation of MSCs into the brain through Hamilton syringe is risky. In this study, we investigated whether the topically applied MSCs could migrate to the injured site in a rodent model of TBI. MSCs derived from the adipose tissue of transgenic green fluorescent protein (GFP)-Spague-Dawley (SD)rats were applied to the external surface of left parietal cerebral cortex of wild-type SD rats (N=10) 2 days after TBI which was induced by a controlled cortical impact (CCI) at the contralateral side. The MSCs were held in position by a thin layer of fibrin glue. Immunohistochemistry staining of GFP showed that 5 days after topical application, the MSCs migrated along the corpus callosum and reached the TBI site on the contralateral cerebral hemisphere. On the other hand, the topically applied MSCs proliferated superficially on the cortex at the application site in the sham-injured control animals without TBI (N=8). No GFP-MSCs were found in the other somatic organs in the test and control animals. Our experiment demonstrated that topical application provides a direct delivery of MSCs to the target organ and the microenviroment of TBI can facilitate the migration of MSCs from the external surface of cerebral cortex to the injury site of the brain.

W-3038

CULTURE OF UROTHELIUM AND MESENCHYMAL STEM CELLS FROM HUMAN URINARY TISSUES

Joelson Tomedi[1], Annelise R. Rosa[2], **Patricia Pranke**[3], Ilma S. Brum[4], Brasil S. Neto[5], Milton Berger[5], Nádya P. Silveira[6]
[1]Post-graduate Program in Material Science; Post-graduate Program in Physiology - Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [2]Post-graduate Program in Material Science; Hematology and Stem Cell Laboratory, Faculty of Pharmacy - Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [3]Post-graduate Program in Material Science; Post-graduate Program in Physiology; Hematology and Stem Cell Laboratory, Faculty of Pharmacy - Federal University of Rio Grande do Sul; Stem Cell Research Institute, Porto Alegre, Brazil, [4]Post-graduate Program in Physiology - Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [5]Department of Surgery, Uro-

logy Division - Hospital de Clínicas, Porto Alegre, Brazil, [6]Post-graduate Program in Material Science; Chemistry Institute - Federal University of Rio Grande do Sul, Porto Alegre, Brazil

Since the first reports on successful engineered urothelial tissue implants at the end of the 20th century, there has been an increasing interest in using reconstructive therapy in patients who suffer irreversible damage to urinary tissues. An effective approach in urologic tissue engineering involves isolation and culture of urothelium and smooth muscle cells for further combination in scaffolds. Although smooth muscle cells have demonstrated the capacity to generate a functional stroma, there has been a growing interest in using mesenchymal stem cells for this purpose. Current thinking is that they can solve the problem of harvesting cells from the donor site, especially when it is severely damaged. This study reports on an approach to isolate urothelium and mesenchymal stem cells from urinary tissues. In addition, the preliminary results of their interaction to temporary matrices prepared with natural biopolymers are presented.
Segments of ureter obtained from nephrectomies provided urothelial cells. At the same time, adipose-derived stem cells (ADSCs) were harvested from a small amount of periureteral and perirenal fat and expanded with Dulbecco´s modified Eagle´s medium (DMEM) supplemented with 10% fetal bovine serum (FBS). A sample of ADSCs was separated in order to perform immunophenotypic characterization and differentiation assay. Following this, each cell type was seeded on scaffolds produced with a combination of gelatin, hyaluronic acid and heparin. The cells and scaffolds were incubated for 2 weeks in DMEM containing 5% FBS. After this period, matrices were fixed in 4% paraformaldehyde and stained with hematoxylin and eosin (HE).
Both cellular types were expanded in primary cultures. Flow cytometry analysis evidenced that ADSCs derived from urinary tissues exhibited a positive expression of CD29 and CD90, and a negative for CD34, CD45 and HLA-DR. Moreover, their adipogenic and osteogenic differentiation potentials were demonstrated in vitro. HE stained paraffin sections of scaffolds showed that urothelial cells were able to form a single layer. On the other hand, ADSCs cultured on temporary matrices developed a multilayer arrangement.
This study reports that human urothelial cells and mesenchymal stem cells can be isolated from urinary tissues in a single procedure. ADSCs obtained from urinary tissues possess immunophenotypic characteristics and differentiation potential similar to those derived from peripheral fat. It was also observed that both cellular types were able to attach and grow in the scaffolds produced. Further studies will evaluate if it is feasible to generate a urothelial mucosa combining both cellular types.

W-3041

HYPOXIA PRECONDITIONING CULTURE ENHANCES BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS MIGRATION AND PARACRINE EFFECTS BY TROPHIC FACTORS.

Min Young Noh, Young Seo Kim, Kyung Ah Cho, Won Jun Choi, Seong-Ho Koh, Seung Hyun Kim
Neurology, Hanyang University College of Medicine, Seoul, Korea, Republic of

Bone marrow-derived Mesenchymal stromal cells (MSC) are emerging candidate cells for the treatment of neurological diseases. However, the majority of MSCs transplanted by hypoxic environment fail to reach the site of injury, and they have demonstrated only minimal therapeutic benefit in clinical trials. Therefore, oxygen tension plays a pivotal role in the migration of MSC. In this study, we aimed to determine whether hypoxia preconditioning could increase the migration capacity of MSCs during *ex vivo* expansion. In MSC cultured at oxygen as low as 1%, rates for cell death and hypoxia-induced gene transcription remained unchanged, while cell migration was significantly increased, western blot analysis revealed increased levels of migration-related signaling proteins such as CXC chemokine receptor 4 (CXCR4). In addition, overexpression of prosurvival genes like Akt can reduced hypoxia-induced cell death, decresed in cell senescence, and exhibit enhanced trilineage differentiation. Hypoxia preconditioning also modulates the paracrine activity of MSCs, causing upregulation of various secretable factors, among which are important angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor-2 (bFGF-2), and angiogenin (ANG). These findings suggest that Hypoxia preconditioning during *ex vivo* expansion of hBM-MSCs may provide a general method of enhancing their engraftment in vivo into a variety of tissues.

W-3042

REDUCTION IN PERICAPSULAR FIBROSIS BY CO-ENCAPSULATION WITH MESENCHYMAL STEM CELLS

Bernard E. Tuch, Vijayaganapathy Vaithilingam, Gail A. McFarland, Penelope A. Bean, Margaret DM Evans
Materials Science & Engineering, CSIRO, North Ryde, Sydney, Australia

Pericapsular fibrotic overgrowth (PFO) needs to be overcome for cellular therapies involving encapsulation in an immunoprotective material to be clinically successful, for example, in the treatment of diabetes. Mesenchymal stem cells (MSC) have innate anti-inflammatory properties and we postulated that co-encapsulation of these with the target cells would reduce PFO. We tested this hypothesis using the human HUH7 cells and co-encapsulated them with either human or mouse MSC in alginate using an air droplet generator to produce microcapsules. Microcapsules were delivered into a xenogeneic model using immunocompetent C57BL6 mice either intraperitoneally (ip) or subcutaneously (sc) and maintained for 21 days. Control groups included empty alginate microcapsules, and microcapsules containing HUH7 cells alone or MSC cells alone. A previously published fibrotic scoring system was used to quantify the degree of PFO following post-mortem retrieval of microcapsules from all test groups.
Data from retrieved intraperitoneal microcapsules showed that PFO for HUH7 cells co-encapsulated with human and mouse MSC in a ratio of 1:1 reduced the fibrotic score by 30% and 35% respectively compared with HUH7 cells alone. Increasing the ratios of mouse MSC:HUH7 to 2:1 and 3:1 further reduced the fibrotic score by 52% and 48% respectively compared with HUH7 alone. When microcapsules were implanted sc rather than ip, PFO for HUH7 cells co-encapsulated with mouse MSC in a ratio of 2:1 was reduced by 54% compared with HUH7cells alone. Taken together, these outcomes showed that the best outcome was achieved when MSC were co-encapsulated with HUH7 in a ratio of 2:1 and implanted sc. However, this outcome did not take into account the formation of a fibrotic pouch around the sc implanted microcapsules although the viability of cells in microcapsules retrieved after 21 days was 90%.
In summary, PFO was reduced to in a challenging mouse model with an active immune system by co-encapsulating mouse MSC with the target cells in a ratio of 2:1 with further benefit obtained by implanting them sc rather than ip.

Pre-clinical and Clinical Applications of Mesenchymal

W-3043

AN IMMUNOTHERAPY STUDY TO COMPARE IMMUNOMUDULATORY OF MESENCHYMAL CELLS DERIVED FROM BONE MARROW AND ADIPOSE TISSUES FOR APPLICATION IN NON-HUMAN PRIMATE KIDNEY TRANPLANTATION

fattah sotoodehnejadnematalahi, Nasser Aghdami, Hossain Baharvand
Royan institute, Tehran, Iran, Islamic Republic of

Multipotent non-hematopoietic progenitor mesenchymal stem cells are originally isolated from bone marrow (BM) as well as adipose tissues and they are capable of differentiating into various mesodermal lineages. Several studies have reported low immunogenicity for MSCs as they appear to have great potential to be used as modulators of immune responses in a variety of diseases related to alloreactive immunity in addition to organ transplantation. In vitro studies have shown immunosuppressive effect of MSCs on T cells, B cells and natural killer cells as well as in vivo prolong skin graft survival. Studies have proposed human adipose tissues (hAD) as an unlimited source of MSCs which showed to have very similar immunological properties to MSCs derived from hBM. As MSCs isolation from BM knows as an aggressive method, therefore in this project we aim to isolate MSCs from adipose tissues and then study in vivo immunomodulatory effect of hAD-MSCs on immune responses in kidney xenograft. In addition, we aim to compare the immune regulation of hAD-MSCs in kidney transplantation in nonhuman primates with hBM-MSCs and identify any possible immunomodulative differences between hAD-MSCs and hBM-MSCs. Understanding of immune modulation consequences by hMSCs is required for their use as derivatives of adipose tissues which provide cells for immunotherapy in clinical applications.

W-3044

MIR-141-3P ACCELERATES HUMAN MESENCHYMAL STEM CELL AGING BY DIRECTLY TARGETING ZMPSTE24

Kyung-Rok Yu
Seoul National Univ, Seoul, Korea, Republic of

ZMPSTE24 is involved in the post-translational maturation step of lamin A. Defects in ZMPSTE24 are associated with the accumulation of prelamin A in the nuclear envelope and lead to premature senescence. However, the signaling pathways that modulate ZMSPTE24 expression levels during adult stem cell senescence have not been elucidated. We found that human mesenchymal stem cells (hMSCs) down-regulated ZMPSTE24 and accumulated prelamin A during replicative or HDAC

inhibitor-induced senescence. The miR-141-3p, which is over-expressed during the senescence process, was able to decrease the ZMPSTE24 expression levels and led to an up-regulation of prelamin A and a DNA damage marker in hMSCs. The transfection of anti-miR-141-3p prevented the reduction of ZMPSTE24 in VPA/SB-treated cells, resulting in the suppression of the induction of abnormal nuclear morphology. In addition, epigenetic histone markers of the chromatin configuration on the miR-141-3p promoter region were transcriptionally activated during senescence. Our findings demonstrate a crucial role of ZMPSTE24 in hMSC senescence.

W-3045

HETEROGENEITY OF SKELETAL PROGENITORS IN MOUSE BONE MARROW.

Maria Rostovskaya, Konstantinos Anastassiadis
Biotec, Dresden, Germany

Bone marrow stroma contains a complex of mesenchymal progenitors generating cells of skeletal tissues, such as osteocytes, chondrocytes and adipocytes. The studies of skeletal progenitors in vivo are hampered by the lack of specific surface markers for their identification in the tissue. On the other hand, a limited proliferative capacity represents a major limitation for the in vitro characterization of those cells.
We generated transgenic mice carrying a modified tetracycline-inducible system for the expression of simian virus 40 (SV40) Large T-antigen depending on the presence of two ligands, Dexamethasone and Doxycycline. Bone marrow mesenchymal stromal cells (BM MSCs) were isolated from the transgenic mice and expanded upon induction of Large T expression.
Conditionally immortalized BM MSCs maintained the capacity to differentiate to osteo-, adipo- and chondrocytes and expressed surface markers characteristic for MSCs. Importantly, the cells did not change those properties after extended passaging. We performed a screening of surface markers of BM MSCs using a panel of 176 antibodies and high-throughput flow cytometry. We found 33 antigens to be expressed, and among them 3 novel for BM MSCs and 13 that have not been reported for MSCs from mice. To our knowledge, this is the most comprehensive characteristic of mouse BM MSC immunophenotype reported to date.
As a next step, we established single-cell derived BM MSC subpopulations by limiting dilutions, and analyzed them for the osteogenic and adipogenic potential. We identified osteogenic, adipogenic and bipotential progenitor types and further checked them for the expression of surface markers by screening with the antibody panel. We found CD200 as a marker of osteogenic lineage and SSEA4 and CD140a as a characteristic of adipogenic cells. Those findings were validated by cellular sorting of BM MSCs followed by the in vitro differentiation.
Furthermore, we characterized transcriptome of tri-, bi- and monopotential BM MSC progenitors using Illumina RNAseq. We identified distinct transcriptional signatures for the osteogenic and adipogenic lineages (all clones that contain “O” or “A” property, respectively), and found that the progenitors with both potentials (“OAC” and “OA”) combine those signatures, and do not express unique genes.
Taken together, we performed an extensive characterization of osteo- and adipogenic lineages in BM MSC progenitors, and identified surface markers and transcription signatures characteristic for these cell types.

W-3046

THE CLINICAL POTENTIAL OF BONE MARROW MESENCHYMAL STEM CELLS (MSCS) CANNOT BE EXTRAPOLATED TO MSCS ISOLATED FROM THE MENSTRUAL FLUID DUE TO MAJOR DIFFERENCES IN THEIR IMMUNOSUPRESSIVE PROPERTIES

Patricia Luz-Crawford[1], Maria-Jose Torres[1], Ainoa Fernandez[2], francisca Alcayaga-Miranda[1], Lorena Salazar[1], Fernando Figueroa[1], **Maroun Khoury**[1]
[1]Faculty of medicine, Laboratory of miRNA and stem cells, Universidad De Los Andes, Santiago, Chile, [2]Universidad Andres Bello, Santiago, Chile

The harvest of mesenchymal stem cells (MSCs) from adult tissues such as the bone marrow or adipose tissue is possible but requires invasive procedures that include discomfort and sometimes complications. Moreover, to reach

clinically effective dosages these sources require a large-scale expansion that might modify their stem-like properties and hence their therapeutical efficacy. A highly proliferative stem cell was recently identified from menstrual blood. The collection of these cells is non-invasive, free of complications and can be repeated periodically in a simplified and reproducible manner that none of the existing sources can match. However, more research is an imperative to analyze their regenerative and immunomodulatory properties.This study focuses at comparatively characterizing the immunoregulatory properties of menstrual stem cells (MenSCs) as opposed to the presently available MSC sources.
In a proliferation assay, MenSCs exerted a significantly less immunosuppressive effect than bone marrow MSCs (BM-MSCs) when cocultured with allogenic activated PBMCs at a 1:10 ratio, (50% and 70% of T cells proliferation respectively and in comparison with the condition without MSCs). Surprisingly, they showed a stimulatory effect on T cells when diluted to a 1:100 ratio (156% of
T cell proliferation) whereas BM-MSCs maintained their suppressive effect (40%). When looking at the different generated T cell subtypes, a significantly higher number of proinflammatory Th1 (11,6% vs 4,5%) and CD8+INFg+ cells (14,1% vs 6,5%) and lower number of Treg and CD4+IL10+ cells(3,5% vs 7%) was observed at a 1:100 ratio when compared to BM-MSCs. Upon INFg and IL-1B stimulation, they showed less secretion and expression of the immunomodulatory molecules PDL-1, IDO, COX-2 and TGFB1. In addition, they showed a dramatic lower surface expression of the IFN-g receptor subunits as well as lower TLR3 and TLR4 levels. In a Xeno-GVHD model, where NOD-SCID Il2rg(-/-) where injected with total human PBMCs, BM-MSCs alleviated the symptoms of GvHD by the immusupression of T cells populations, in contrast, MenSCs had no therapeutical benefit. In conclusion, we demonstrate that MenSCs do not possess the immunosupressive potential when compared to BM-MSCs due to the low expression level of many factors involved in this mechanism. The need of this type of information is decisive with respect to the development of safe and effective cell therapies for immune mediated diseases as many autologous and allogenic clinical trials using these cells are being planned.

W-3047

PROSPECTIVE ISOLATION AND CHARACTERIZATION OF MULTIPOTENTIAL MESENCHYMAL STROMAL CELLS AND COMMITTED OSTEOPROGENITOR CELLS IN MOUSE BONE MARROW

Ching fang Chang[1], Ke hsun Hsu[1], Jhih jia Liao[2], Chia Yi Yang[2], Chung leung Li[3], Jean Lu[2]
[1]Institute of Microbiology and Immunology, National Yang-Ming University, Taipei city, Taiwan, [A]Stem Cell Program, Institute of Genomics Research Center, [B]Stem Cell Program, Institute of Cellular and Organismic Biology/Genomics Research Center, [2]Academia Sinica, Taipei city, Taiwan

Currently multipotential stromal/mesenchymal stem cells (MSCs) are defined mostly on their in vitro differentiation capacity and cell surface marker profile. However whether these characterization parameters are physiologically relevant remains unclear and should be critically addressed. To further complicate the issue, MSCs are mostly studied as a heterogeneous population of cultured adherent cells and even if the differentiation potential is measured at clonal level, the initiating cells have already undergone a minimum of 20 population doublings, so it is difficult to determine whether the observed multipotential differentiation potential truely reflect that of the original initiating cell or has been acquired during culture expansion. In an attempt to clarify the above issues, we chose to use FACS to isolate different cell populations based on their differential expression of Sca-1, CD24, and PDGFR-α (Pα) antigen within the (CD31/CD45/Ter119)- marrow stromal cell fraction. Based on the results of in vitro differentiation assays performed on the short-term culture expanded cells and primary colony assays performed on fresh isolated cells, the Sca-1hi, Sca-1lo and Sca-1- cell fractions are enriched for MSCs, committed osteoprogenitors and mature ALP+ osteogenic cells respectively and we propose a hierarchical stepwise differentiation model for the osteoblast cell lineage within the marrow. More complete data including (i) in vivo ectopic bone formation and osteogenic reconstitution assays will be presented to further substantiate the proposed model. We believe that this pivot study will provide a strategy and platform to investigate the function and developmental relationship of MSC and the osteoprogenitors in vivo in the near future.

W-3048

GENE EXPRESSION ANALYSIS OF AGING HUMAN MULTIPOTENT STROMAL CELLS

Ian H. Bellayr[1], Jennifer G. Catalano[1], Samir Lababidi[2], Amy X. Yang[1], Jessica L. LoSurdo[1], Steven R. Bauer[1], Raj K. Puri[1]

[1]CBER, Office of Cellular, Tissue and Gene Therapies, FDA, Bethesda, MD, United States, [2]CBER, Office of Biostatistics and Epidemiology, FDA, Rockville, MD, United States

Stem cell based therapies have tremendous potential in the field of regenerative medicine to treat human disorders, such as organ failure, traumatic limb injuries, and genetic disorders. Multipotent stromal cells (MSCs) isolated from bone marrow can be expanded in vitro and have the potential to differentiate into osteogenic, chondrogenic and adipogenic cell lineages. In addition, as MSCs have inherent characteristics to suppress the immune system, they are also being studied in preventing graft-versus-host disease. However, MSCs have not been fully characterized by unique markers that can define their quality and thus predict their safety and effectiveness. Moreover, the impact of growth characteristics, donor variability, age and the sex of the donors has not been fully realized on the function and identity of these cells. The objective of this study was to identify genes that were associated with aging by cell passage. Human MSCs from 8 different donors, grown under identical culture conditions and harvested at different cell passages (3, 5, and 7), were analyzed by high-throughput microarrays. Total RNA with an average RNA integrity number (RIN) of 9.9 ± 0.16 was isolated from each sample. Microarrays containing 35,000 70-mer oligonucleotide probes were produced in-house at Food and Drug Administration, Center for Biologics Evaluation and Research. Dual dye labeled cDNA was hybridized to the microarray overnight where a common reference RNA composed of equal aliquots of passage 3 RNA from 6 donors was employed. All samples were run in triplicate by a randomized block design. An Analysis of Variance with multiple testing corrections found that 34 probes were statistically significant with 12 of them having an unknown function. The 22 known genes were imported into ingenuity pathway analysis software and analysis of the top scoring networks ($p<0.05$) had the functions of carbohydrate metabolism, small molecule biochemistry and cellular development. Four of the known genes were found to have a statistically significant trend with increasing passage. It is expected that the results obtained from this study will identify molecular markers that improve the ability to predict MSC performance. The identification of such biomarkers will facilitate the clinical development of MSCs in regenerative medicine.

W-3051

THE MRNA STABILIZING PROTEIN, CRD-BP/IMP1 AND TET FAMILY MEMBERS COOPERATE TO REGULATE HUMAN MESENCHYMAL STEM CELL PROLIFERATIVE CAPACITY.

LOUISA MAHAIRA, **OLGA KATSARA**, CONSTANTIN BAXEVANIS, MICHAEL PAPAMICHAIL, SONIA PEREZ
SAINT SAVAS, CANCER IMMUNOLOGY AND IMMUNOTHERAPY CENTER, ATHENS, Greece

Mesenchymal Stem Cells (MSC) is a population that can be found in many tissues and can differentiate towards many different lineages. Unraveling the molecular profile of MSC is of great importance, due to the fact that these cells are very often used in preclinical and clinical studies. We have previously reported the expression of CRD-BP/IMP1- an oncofetal mRNA binding protein- in different stem cell populations, specifically bone marrow (BM)-MSC and umbilical cord blood- hematopoietic stem cells (UCB-HSC), and have attributed to it an important role in stem cells with high self-renewal capacity. Now we demonstrate for the first time that MSC of adipose tissue (AT), BM, and UC origin have a differential pattern of CRD-BP/IMP-1 expression that could correlate with their proliferation rate- since gene knocking down resulted in significant reduction of cell proliferation and self renewal. We also provide evidence for the involvment of TET family members in the epigenetic regulation of CRD-BP/IMP-1 in human MSC; and show that TET1, TET2, CRD-BP/IMP1 along with c-MYC are pivotal parts of a complex network involved in the regulation of MSC proliferative capacity.

W-3052

STEM CELL FROM THE VOMERONASAL ORGAN: POTENTIAL FOR USE IN CELL THERAPY

Marcio N. Rodrigues[1], Rafael C. Carvalho[2], Dayane Alcântara[1], Graciela C. Pignatari[1], Káterin E. B Grondona[3], Maria A. Miglino[1]

[1]Surgery, University of Sao Paulo, Sao Paulo, Brazil, [2]Federal University of Maranhao, Chapadinha, Brazil, [3]Center of Agricultural Science, Federal University of Paraiba, Areia, Brazil

The vomeronasal organ (VNO) is located at the base of the nasal cavity, has a tubular shape, is divided by the nasal septum, and forms part of the olfactory pathway. It emits chemical signals that detect pheromones and modulate social and reproductive behavior. The VNO has stem cells adjacent to the basal lamina, able to divided and migrate to replace sensory neurons, these stem cells can generate functional neurons throughout animal life. The aim of study was to isolate and characterize cells derived from the vomeronasal organ of New Zealand rabbits. Five male rabbits were used for collection of the VNO explants, and three culture media were tested (α-MEM, DMEM-F12, and High-Glucose). Cells were possible to be isolated in all media tested. However there was a greater confluence and cell growth when was used the High-glucose media, confirmed by the proliferation assay (MTT). In immunocytochemistry positive staining was observed for CD105, CD90, CD 73, Nanog, Vimentin, Cytokeratin 18, beta-tubulin, nestin, GFAP, PCNA, Oct-4, and Stro-1, negative reactions was observed for CD45, CD34 and CD117. In flow cytometry, they were negative for CD45 and CD34 and positive for Nanog (34.6%), CD 105 (46.6%), CD 90 (48.4%), Oct ¾ (45.1%) and Stro-1 (48%). Cultured cells were able to functionally differentiate into adipocytes, osteocytes and chondrocytes nor carninoma was formed when performed the assay in nude mice. It was possible to verify that the cells could be isolated in all three protocols proposed and they provide security for cellular therapy, since there was no formation tumorigenic.

W-3053

COMPARATIVE EVALUATION OF OCT-4, SOX2 AND C-MYC EXPRESSIONS IN HUMAN DENTAL PULP STEM CELLS VS. HUMAN DENTAL FOLLICLE STEM CELLS

Razieh Karamzadeh[1], Mohamadreza Baghaban Eslaminejad[1], Reza Aflatoonian[2]

[1]Department of Stem Cell and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran, Islamic Republic of, [2]Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran, Islamic Republic of

Background:
Induced pluripotent stem cells (iPSCs) technology has been open a promising place in the field of regenerative medicine by providing the transformation of somatic cells into iPSCs with four core transcription factors, Oct-4, Sox2, c-Myc & Klf4. Recently, it has been shown that these transcription factors express in human dental pulp stem cells (hDPSCs), which may involve in maintaining stem cells self-renewal capacity. Lately, there is a subtype of dental stem cells known as dental follicle stem cells (hDFSCs), which shows mesnechymal stem cells (MSCs) features & differentiation potentials into mesodermal and/or non-mesodermal lineages. According to the different types of stem cells in dental tissues & their various differentiation capacities, we investigate the expression patterns of Oct-4, Sox2 & c-Myc in hDPSCs compare to the hDFSCs.
Methods:
hDPSCs & hDFSCs were enzymatically isolated from wisdom teeth (n=3) and characterized in terms of surface epitopes (MSC markers CD90/CD73/CD44/CD105, hematopoietic/endothelial markers CD34/CD45/CD11b/CD31 & Pre-vascular marker CD146) as well as differentiation capacity into bone, cartilage & adipose cells. Then, these two types of cells were undergone to comparative assessment of Oct-4, Sox2 & c-Myc expressions by QPCR & Immunofluorescence.
Results:

hDPSCs & hDFSCs showed MSC phenotype & differentiation capacity into three mesenchyme lineages. Immunophenotyping results confirmed the existence of MSC markers and the lack of hematopoietic/endothelial markers in both groups. The results of QPCR & Immunofluorescence indicated the expressions of Oct-4, Sox2 & c-Myc genes in both hDPSCs & hDFSCs. Interestingly, these results showed significant higher expression of Sox2 & c-Myc genes in hDPSCs in comparison with hDFSCs. In contrast, Oct-4 gene expression was more in hDFSCs compare to the hDPSCs. These results may show distinct role of Oct-4, Sox2 & c-Myc, as the core reprogramming factors, in these cells.

W-3054

IS SUBSTANCE P A CHEMOTACTIC FACTOR TO ATTRACT STEM CELLS TO THE DYSTROPHIC MUSCLE?

Amanda F. Assoni, Natássia M. Vieira, Mariane Secco, Marcos Valadares, Juliana PA Gomes, Mayra Pelatti, Mayana Zatz
INSTITUTO DE BIOCIENCIAS, UNIVERSIDADE DE SÃO PAULO, São Paulo, Brazil

Progressive Muscular Dystrophies (PMD) are a clinical and genetic heterogeneous group of diseases characterized by a progressive and irreversible degeneration of skeletal muscles with variable distribution and severity. The therapeutic potential of mesenchymal stromal cells (MSCs) from different sources such as bone marrow, umbilical cord tissue, adipose tissue and dental pulp has been thoroughly investigated for PMDs. However, to date, none of the pre-clinical trials involving cell therapy in different animal models for PMDs showed a complete success (restoration of both absent or defective protein and functional recovery). Therefore, enhancement of both cell homing and differentiation will be of great importance in an attempt to obtain a more successful cell therapy for PMDs. Different soluble factors released by cells present at sites of injury may attract and stimulate MSCs population. Recently it was reported that substance P (SP) acts as chemotactic for MSC after injury. SP is a neurotransmitter that is associated with neurokinin-1(NK-1), producing different responses such as vasodilation, plasma protein extravasation in post capillary venules and leukocytes adhesion on endothelial cells of venules. Here we evaluated the expression of SP in the dystrophic muscle of different PMDs murine models (dy2J, SJL, mdx, as compared to C57B6 and Swiss as background controls), quantified its receptor, NK-1, in different sources of MSCs(bone marrow, umbilical cord, adipose tissue, periosteum, palate, dental pulp, menstrual blood, endometrium, fallopian tubes, orbicularis oris muscle, as compared to fibroblasts which were used as controls) and correlated its expression with its potential as a chemotactic factor. Thirty-four (6-7 per group) animals were used for quantification of gene and protein expression of SP. To quantify NK-1, 70 lineages of MSCs (5-7 per group) were analyzed into passage 4.The results demonstrated great variability in the amount of SP in the muscles of the murine models within the lineages . Interestingly a greater amount of SP was observed in muscles from the mdx mice, as compared to normal controls, a finding which is currently being further investigated. Among the different sources of MSCs, wide intra variability in NK-1 amount was also observed. However, stem cells derived from fat showed in general a higher quantity, which may be important for choosing the best source aiming a possible therapy. The experiments to correlate the amount of SP receptor with the chemotactic potential of SP over MSCs are currently underway.

W-3055

CARTILAGE STEM / PROGENITOR CELLS : CHARACTERIZATION AND ROLE IN OSTEOARTHRITIS

Yangzi Jiang[1], Changchang Hu[2], Wei Zhang[4], Shufang Zhang[5], Ping Lu[3], Dan Yang[7], Zi Yin[6], Linlin Wang[8], Rocky S. Tuan**[1], Hongwei Ouyang*[3]
[1]Department of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA, United States, [2]School of Medicine, Zhejiang University, Hangzhou, China, [B]Zhejiang Provincial Key Laboratory of Tissue Engineering and Regenerative Medicine, [2]School of Medicine, Zhejiang University, Hangzhou, China, [3]Zhejiang Provincial Key Laboratory of Tissue Engineering and Regenerative Medicine, Hangzhou, China, [A]Center for Stem Cell and Tissue Engineering, [B]Zhejiang Provincial Key Laboratory of Tissue Engineering and Regenerative Medicine, [4]School of Medicine,Zhejiang

University, Hangzhou, China, [5]Hangzhou Longhill Bio-medication Technology Co.,Ltd, Hangzhou, China, [6]School of Medicine,, Zhejiang University, Hangzhou, China

Abstract

BACKGROUND: Tissue specific stem cells have been recently identified in osteoarthritic (OA) cartilage tissues on the basis of their migratory properties. However, the characteristics and biological activities of the Cartilage Stem/Progenitor Cells (CSPC) in OA are not clearly known.

AIM: This study aims to study the phenotype markers of CSPCs during in vitro amplification and analyze their relationship with OA development and therapy.

METHODs: Cartilage biopsy was harvested from human OA and normal knee joints with institutional approval. CSPCs were isolated by clonal selection and expanded in low density culture. Cell phenotype was evaluated with immunofluorescence, flow cytometry, and qRT-PCR. The CSPC subpopulation was selected by FACS. Osteo- and adipogenic differentiation was evaluated by Alizarin Red and Oil-red O staining, respectively, and chondrogenic differentiation was analyzed in pellet cultures.

RESULTS: CSPCs were isolated from 30 OA and 10 normal cartilage samples. A dynamic temporal profile of the phenotype markers, CD146 and collagen type II (Col2), was seen - Col2 was positive after isolation and became negative after passage 3, while CD146 became positive with passage from ~30% in P1 to ~80% in P3. IL-1β treatment increased cell proliferation of normal CSPCs and upregulated endogenous IL-1βexpression, but did not affect the percentage of CD146+ cells. FACS-sorted CD146+ CSPCs showed 2-fold higher clonal forming ability compared to CD146- cells, suggesting that CD146 may be a biomarker for CSPCs. Interestingly, some sorted CD146- cells transitioned into CD146+ cells after monolayer culture. Expression profiles of several genes related to self-renewal and multipotency regulation (actin fliament-associated protein, Frizzled 7, Dickkopf 3, protein tryosine phosphatase receptor F, and RAB3B) also changed with passage, RAB3B was upregulated and the PTPRF/FZD7/DKK3/AFAP was downregulated. Compared to human bone marrow stromal cells (BM-MSCs), CSPCs exhibit multiple linage differentiation ability, but are less osteogenic and adipogenic. After 4 weeks in chondrogenic culture, BM-MSC pellets retained some fibroblast like phenotype in the center, but CSPC pellet consisted largely of cells with chondrocyte like phenotype. The cells in CSPC pellets were Col2+ and CD146-, but became CD 146+ again when they migrated away from the pellets and subsequently cultured in monolayer.

CONCLUSIONS: These findings demonstrated the kinetic and biological profile of the phenotype of CSPCs, and implicated their alterations and possible roles during OA pathogenesis.

(Support: Commonwealth of PA Dept of Health)

W-3056

ENHANCING EX-VIVO EXPANSION OF CORD BLOOD-DERIVED UNRESTRICTED SOMATIC STEM CELLS FOR CLINICAL APPLICATIONS

Hanan G. El-Baz[1], Zeinab A. Demerdash[1], Faten S. Mahmoud[1], Salwa H. Mohammad[1], Kesmat M. Maher[1], Dina S. Abd El Hady[1], Marwa H. Ahmad[1], Tamer F. Taha[2]

[1]Immuonology Departement, Theodor Bilharz Research Institute, Cairo, Egypt, [2]Reproductive Health Departement, National Research Center, Cairo, Egypt

ABSTRACT

BACKGROUND: Cord blood (CB) derived Unrestricted Somatic Stem Cells (USSCs) hold great promise as therapeutic agents in regenerative medicine and autoimmune diseases. Ex-vivo expansion of USSCs is a prerequisite for evaluating their therapeutic potential in ongoing clinical trials. This study was designed to determine factors (such as: seeding density, culture media and growth supplements) driving USSCs population to expand extensively to generate cell number suitable for clinical application.

STUDY DESIGN AND METHODS: Isolation, propagation and characterization of USSCs from CB samples were performed. The influence of log fold decreased USSCs seeding (5000, 500, 50 and 5 cells /cm2) in three culture me-

dia; 30% FBS/DMEM, 2% FBS/EGM-2, and serum free media (Mesencult), on clinical-scale expansion of USSCs, was evaluated.
RESULTS: USSCs isolated from 9 out of 12 CB samples (75%) showed the classical MSCs morphology of adherent fibroblastoid spindle-shaped cells growing in a monolayer. USSCs expressed high levels of CD44, CD90 CD73 and CD105, whereas they were negative for CD34, CD45, and class II (HLA-DR). They showed high expression of transcripts for Oct4 and Sox2 which are the core transcription factors in early embryo development and pluripotency maintenance in embryonic stem cells. In addition, they were induced to differentiate into osteoblasts and adipocytes. USSCs cultured in 30% FBS/DMEM showed significantly higher Population Doubling (PD) ($p<0.001$) than those cultured in EGM-2 or Mesencult at all cell densities. PD of USSCs cultured at 5 cells /cm2 in either 30% FBS/DMEM, or EGM-2, was significantly higher ($p<0.001$) than those cultured at higher densities. However, at a higher cell density (50 cells /cm2) PD of USSCs cultured in Mesencult was significantly increased ($p<0.001$) than that of other densities.
CONCLUSION: On the basis of optimizing cell culture conditions of USSCs to scale up their number to a high level suitable for clinical applications, the following two step scenario is recommended; Step one: Culturing of CB-derived USSCs of early passages (P1-P3) in 30% FBS/DMEM at low cell seeding density (5 cells /cm2) to allow for scaling up of USSCs count with less passaging frequency, to avoid the unwanted effects of repeated trypsinization. Second step: Culturing of the expanded USSCs (>P3) in Mesencult serum free medium at a higher cell seeding density (50 cells /cm2), to avoid the undesirable effects of FBS on cultured cells. The efficiency of this two-step procedure for clinical-scale USSCs propagation may facilitate rational clinical testing of USSCs-based therapies.

Mesenchymal Stem Cell Differentiation

W-3057

BIOMIMETIC NICHE DESIGN FOR GROWTH FACTOR MEDIATED DIFFERENTIATION OF HUMAN ADIPOSE DERIVED MESENCHYMAL STEM CELLS TO CARDIAC LINEAGE

Subha S
Thrombosis Research Unit, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, India

Myocardial infarction (MI) is a leading cause of morbidity and mortality worldwide. Regeneration of damaged cardiac tissue involves generation of cardiomyocytes (CMs) and other supporting cells. Out of all cells essential for cardiac regeneration, CM is most important for retaining the contractile and electrical activity of heart muscle. Replication of CM is difficult as they are terminally differentiated cells. Mobilization, homing and differentiation of cardiac stem cell is not achieved due to poor milieu conditions after MI. Cardiomyoplasty using various cell sources including embryonic stem cells, induced pluripotent stem cells and adult stem cells have reported encouraging results but unsatisfactory clinical outcome. Differentiation of stem cells to cardiac lineage may be achieved using culture matrix immobilized with chemokines and cytokines. Polymerized fibrin can retain growth factors to produce bio mimetic culture matrix; however, cell-specific composition may be needed for each cell type. The objective of this study is to constitute a fibrin-based bio mimetic matrix for lineage commitment of adipose derived mesenchymal stem cell (ADMSC) to enable potential use of adipose tissue as autologous source for regenerative therapy in MI patients.
Adipose tissue was collected from human patients during cardiac surgery with institutional ethics committee's approval. Cells released upon collagenase treatment were cultured using standard published protocol. Cells from third passage were characterized using panel of 3 positive (CD105, CD44, CD90) and 2 negative (CD34 and CD45) surface markers. Thin layer of fibrin matrix was prepared by clotting fibrinogen concentrate on thrombin adsorbed surface. The bio mimetic matrix of the niche comprised fibrin, fibronectin, gelatin and growth factors (GFs) such as bone morphogenetic protein-4 (BMP-4) vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF). Culture- expanded, homogenous ADMSCs were seeded onto the matrix. Low glucose DMEM supplemented with fetal calf serum and GFs was used to grow the cells. Graded concentrations of BMP-4, VEGF and FGF were tried out to identify a composition that produced maximum number of CMs. Nanogram level of GFs per 2 mg fibrino-

gen concentrate constituted a matrix composition which induced morphological changes to ADMSCs, suggesting lineage commitment into CMs. Immunological and genetic markers were used to characterize CM. Cardiac lineage markers Flk1, N-cadherin and c-Kit indicated matrix directed differentiation; percentage of CM was found to be low in flow cytometric analysis. Differentiation of MSCs into cardiac lineage was confirmed by studying the expression levels of cardiac specific transcription factors including Nkx2.5 and GATA-4.
Conclusion: Combination of adhesive matrix proteins and growth factors at a specific composition induced differentiation of ADMSC into cardiac lineage in vitro. More optimization is required to improve the yield of CM precursors for potential application for MI therapy using autologous adipose tissue.

W-3058

COMPARISON BETWEEN IN VITRO DIFFERENTIATIONS OF ADIPOSE DERIVED MESENCHYMAL STROMAL CELLS FROM TWO DIFFERENT SOURCES: OUTER THIGH AND ABDOMEN

Juliana P. Gomes, Natássia M. Vieira, Marcos C. Valadares, Amanda F. Assoni, Mayra Pelatti, Mariane Secco, Mayana Zatz
University of São Paulo, São Paulo, Brazil

We have previously observed that adipose (ADMSC) and umbilical cord derived mesenchymal stromal cells have different behavior when injected in a dystrophic mouse model, which could be explained by the intimacy between the cells and their niche - or microenvironment. On the other hand, we and others have found that adipose tissue is a rich source of MSCs. Therefore, the aim of the present study was to verify if ADMSC from different niches differ in their in vitro potential to differentiate in specific cell lines or maintenance of stem cell multipotency. In order to circumvent individual variability we have obtained two ADMSC paired samples from nine unrelated donors: right or left outer thigh and abdomen. These samples were analyzed for their in vitro chondrogenic, adipogenic, myogenic and osteogenic differentiation potentials, during a 21 day differentiation protocol. Specific commercial inducing medium (Invitrogen) was used and the induced samples were compared with their negative control, which was cultivated with non-inductive medium DMEM Low Glucose. Myogenic differentiation was evaluated on days 0, 8 and 15 of differentiation by Real Time PCR of dystrophin and myosin encoding genes expression and also by Western Blot and immunohistochemistry. For adipogenic differentiation both quantitative (AdipoRed) and qualitative (Oil Red) colorimetric test were applied on day 21. Chondrogenic differentiation was analyzed by Toluidine Blue staining. Osteogenic potential was evaluated by alkaline phosphatase activity on day 10 of differentiation and by Alizarin Red staining on days 14 and 21. The Alizarin Red staining was extracted from the plate with an alcoholic solution and was quantitatively measured in a spectrophotometer. No differences were observed for myogenic, adipogenic or chondrogenic differentiation between ADMSC from the two sources. However, Alizarin Red staining on day 21 revealed a statistically significant difference for osteogenic differentiation in samples obtained from thighs as compared to abdomen (Mann-Whitney test; $P < 0.05$). These observations suggest that ADMSC from outer thigh have a better osteogenic potential than ADMSC from abdomen. Aiming to validate these results, studies by Real Time PCR for expression of genes related with osteogenic in vitro differentiation as well as in vivo experiments are in progress.

W-3061

EVALUATING THE EFFICACY OF ENDOTHELIAL CELLS DIFFERENTIATED FROM ADIPOSE DERIVED MESENCHYMAL STEM CELLS FOR IN VITRO INTIMA CONSTRUCTION

A. Eser ELCIN[1], Y. Emre ARSLAN[2], Y. Murat ELCIN[2]
[1]Ankara University Stem Cell Institute & Faculty of Science, TEBNL; Gazi University GEF, Ankara, Turkey, [2]Ankara University Stem Cell Institute & Faculty of Science, TEBNL, Ankara, Turkey

Adipose tissue is an attractive candidate to become a major stem cell source for regenerative medicine and tissue engineering applications. Adipose-derived mesenchymal stem cells (AdMSCs) possess multilineage differentiation potential. Differentiation into mesodermal cell types, i.e. into adipocytes, chondrocytes, osteoblasts, and myo-

cytes has been shown in several studies. They also can be induced to differentiate into ecto and endodermal origin, e.g. hepatocytes, pancreatic islet cells, neural cells, epithelial cells, and endothelial cells (ECs). The objective of this study was to investigate the differentiation capacity of human (h) AdMSCs into vascular endothelial-like cells, and to evaluate the efficiency of their use for in vitro construction of a functional tunica intima layer into an engineered-vascular scaffold. Methods. hAdMSCs were isolated from human lipoaspirates, expanded, then immunophenotypically characterized by FACS and by their tri-lineage differentiation potential. Passages 4-6 were used in all experiments. AdMSCs were seeded on collagen type IV-coated surfaces and cultured for 7 and 14 days in the EC-GM containing FGF2, VEGF, heparin, IGF-I, EGF, hydrocortisone, and ascorbic acid. hAdMSCs were also seeded inside a nanofibrous tubular polymer scaffold and differentiated into ECs either in static or flow conditions. As positive control, human internal mammary artery endothelial cells (hIMAECs) were isolated and expanded on bovine skin gelatin-coated tissue culture plates in EC proliferative medium (EC-PM); hAdMSCs cultured in standard medium without differentiation inducers served as the negative control in all experiments. To evaluate EC characteristics, immunohistochemistry studies were performed using antibodies against six EC-specific proteins. Additionally, acetylated-low density lipoprotein (ac-LDL) uptake and expression of endothelial cell nitric oxide synthase (eNOS) were determinated for endothelial cell function analysis. Results: Human AdMSCs had CD 90+, CD105+, CD73+, CD29+, CD44+, CD34-, CD45-, and CD133- immunophenotype and demonstrated tri-lineage differentiation potential. Immunohistochemistry demonstrated that hAdMSCs on nanofibrous tubular construct differentiated into endothelial-like cells, and expressed CD31 (PECAM-1), CD34, von Willebrand factor (vWF), VE- cadherin (CD144), tie-2 and flk-1 (VEGFR2) at day 7. Overall, the expression levels of these endothelial specific proteins significantly increased after 14 days which were comparable with that of the hIMAECs. While the expressions of eNOS and ac-LDL uptake were found to be quite low in static culture, a positive influence was observed under in vitro biomimetic shear conditions. Conclusion. Human adipose mesenchymal stem cells can be differentiated into functional vascular endothelial-like cells inside a nanofibrous tubular scaffold under flow conditions that express EC-specific proteins. Findings support the notion that the tunica intima can be constructed in vitro using ECs derived from hAdMSCs. Currently, we are testing the possibility of increasing expression levels of eNOS and ac-LDL uptake by constructing a media layer containing smooth muscle cells in close contact with the neointima layer inside a a real time computer-controlled bioreactor system.

W-3062

TRANSPLANTATION OF ADIPOSE TISSUE DERIVED MULTI LINEAGE PROGENITOR CELLS VIA PORTAL VEIN IMPROVED SERUM LEVELS OF BETA GALACTOSIDASE IN GM1 GANGLIOSIDOSIS MODEL MICE.

Mayumi Soeda[1], **Hanayuki Okura**[1], Junichiro Matsuda[2], Minako Koura[2], Mitsuko Morita[1], Junko Tani[1], Akihiro Ichinose[3], Akifumi Matsuyama[1]

[1]Foundation for Biomedical Res & Innovation, Kobe, Japan, [2]Laboratory of Animal Models for Human Diseases, National Institute of Biomedical Innovation, Osaka, Japan, [3]Kobe University Hospital, Kobe, Japan

Background: GM1 gangliosidosis and Morquio B are autosomal recessive storage disorders caused by the deficiency of β-galactosidase (GLB1). We have reported that adipose tissue-derived multi-lineage progenitor cells (ADMPCs) could reprogrammed into hepatocyte-like cells in situ and the cells have improved the serum cholesterol levels of familial hypercholesterolemia model Watanabe heritable hyperlipidemic rabbit. Here we examined whether ADMPCs could ameliorate the GLB1 levels of GM1-gangliosidosis model GLB1-knockout mice. Methods and Results: GLB1 expressions of ADMPCs were examined by mannose-6-phospahte (M6P) and mannosamine competition release into cultured media. Incubation with them revealed GLB1 secretion into cultured media in dose dependent manner, resulted in ADMPCs express GLB1. Next, GLB1-knockout mice were received normal mice-derived ADMPCs (n=6) or lactic control (n=3), via portal vein and followed for 4 weeks, and then the serum levels of GLB1were examined by beta-galactosidase enzymatic activities. The serum levels of GLB1 were 7.1 mU of beta-galactosidase (normal control mice, n=6), 0.46 (GLB1-knockout mice treated with lactic control, n=3) and 3.54 (GLB1-knockout mice treated with ADMPC, n=6), respectively. There results indicated the ADMPCs transplantation could improve GLB1 deficiency in GM1-gangliosidosis model mice. To confirm in situ survival of the ADMPCs in hepatic milieu,

GFP-mice-derived ADMPCs (GFP-ADMPCs) were transplanted via portal vein and their liver specimens were applied for histological analysis 4 weeks later. As reported ADMPCs were survived in hepatic milieu and reprogrammed into albumin-expressing hepatocyte-like cells. Conclusion: These results indicated that the transplantation of ADMPC via portal vein is a potentially effective therapeutic strategy for GM1-gangliosidosis.

W-3063

NEW DEFINED CELL CULTURE CONDITIONS IN COMBINATION WITH A 3D-SCAFFOLD FOR ASCS BONE TISSUE ENGINEERING

Mattia Corazza[1], Greta Minonzio[1], Luca Mariotta[1], Mauro Gola[2], Gianni Soldati[1]
[1]Swiss Stem Cell Foundation, Lugano, Switzerland, [2]Stem Cell Research, Lugano, Switzerland

Introduction. Skeletal tissue loss due to congenital defects, disease or injury is normally treated by autologous tissue grafting. This method is limited by the low availability of host tissue, harvesting difficulties, donor site morbidity and clinician's ability to manipulate delicate 3D shapes.
The generation of autologous bone grafts in vitro is the ultimate goal in bone tissue engineering because it avoids the harvesting of autologous tissue from a second anatomic location. To achieve this, three major components have to be developed and optimized: I) cell culturing in a defined medium, II) the osteoinductive cocktail and III) the scaffold.
Results. We developed a protocol for the extraction and culturing of Adipose tissue-derived multipotent Stromal Cells (ASCs) which fulfils the strict European regulations concerning the Advanced Therapy Medicinal Products. ASCs were grown inside a 3D-scaffold (Osteoxenon) developed by BIOACTIVA S.r.l (Italy). This scaffold is based on decellularized equine bone grafts. ASCs were obtained from liposuction aspirates, cultured and expanded using a protocol developed in our laboratory. After seeding the ASCs into the scaffold, the cells were cultured for three weeks in the presence of our new defined xeno-free osteogenic induction medium.
After fixation of the cells with standard techniques, the scaffolds were sliced and stained. The sections were examined by electron microscopy in order to evaluate the cell morphology, spreading and adhesion properties. The ability of the cells to properly differentiate was explored using standard techniques (histology and PCR) on early or late osteogenic markers like RunX2, SPP1, TW1.
Conclusions. Here we show that the scaffold "Osteoxenon", in combination with our defined culture conditions, is suitable for the growth and differentiation of ASCs into osteogenic cells. Furthermore, the bone tissue engineering strategy described here appears to be very promising for the development of protocols for the treatment of bone loss and injury. In the future, the performance of our in vitro generated autologous bone graft will be studied in a human clinical study.

W-3064

PURIFICATION AND DIFFERENTIATION OF HUMAN ADIPOSE DERIVED STEM CELLS THROUGH SILK SCREEN/PLGA HYBRID MEMBRANES BY MEMBRANE FILTRATION METHOD

Li-Yu Chen, Yi-Wei Huang, Meng-Hsueh Wu, Chia-Ju Hsu, Chia-Yi Lin, Akon Higuchi
Department of chemical and Materials Engineering, National Central University, Jhongli City, Taiwan

Human adipose-derived stem cells (hADSC) represent one of the most promising cell candidates in the field of regenerative medicine, it can easily be extracted in large amount compared to bone marrow stem cells and also exhibited a higher proliferation rate in the medium. Furthermore, hADSCs also have a great differentiation capability into mesoderm (muscle, bone, and cartilage) and ectoderm (nerves and epidermal). The cultivation of adipose tissue cells contains adipose stem cells with several other types of cells, so it is necessary to purify hADSCs before using them for clinical applications. Purifying hADSCs via the culture process requires 5-12 days by using the conventional culture method. We developed a membrane filtration method to purify hADSCs using novel membranes where the operation time is less than 30 min to purify hADSCs from adipose tissue solution in this study. We prepared silk screen/PLGA hybrid membranes (scaffolds) by a freeze drying method where silk screens (170 mesh size)

are natural and readily biodegradable protein fibers to be used reinforcement of the membranes and PLGA has biodegradability and biocompatibility to be used to generate a sponge pore morphology of the membranes. The average pore size of the silk screen/PLGA hybrid membranes was measured to be 3.5-7µm from scanning electron microscopy. After the preparation of home-made silk screen/PLGA hybrid membranes, we investigated the purification of hADSCs from adipose tissue solution (adipose tissue-derived stromal vascular fraction [SVF]) having different cell density by the membrane filtration method and compared the purification efficiency of hADSCs. The mesenchymal stem cell (MSC) markers such as CD44, CD73, and CD90 expressed by hADSCs were less than 10% in the adipose tissue solution (SVF), while the MSC markers in the permeate solution were found to be 30-50%, indicating hADSCs were concentrated after permeation through the silk screen/PLGA hybrid membranes, when 9 ml of the adipose tissue solution having 1x106 cells/ml was permeated through the membranes. The MSC markers of the cells after 12 days of culture of the adipose tissue solution (hADSCs purified by the conventional culture method) were found to be 60-80%. The efficiency of hADSC purification in the permeate through the silk screen/PLGA hybrid membranes analyzed by MSC markers depended on the cell density of the adipose tissue solution. Currently 1x106 cells/ml was the optimal cell density compared to 2x106 cells/ml or 4x106 cells/ml when 9 ml of adipose tissue solution was used. It was demonstrated that more than two fold higher osteogenic gene expression, Alizarin red staining, and von Kossa staining was observed in the permeate solution compared to the adipose tissue solution (SVF) when the cells were cultured in osteogenic induction medium for 28 days. Therefore, the hADSCs were purified in the permeation solution demonstrated a superior capacity for osteogenic differentiation than the cells in the adipose tissue solution (SVF). The polyurethane foaming membranes having pore size of 11 µm could not purify hADSCs in the permeate solution. This result indicates that the pore size and membrane material are important factors to purify the hADSCs by the membrane filtration method. It is concluded that the hADSCs can be easily isolated through the permeation through the silk screen/PLGA hybrid membrane, whereas non-hADSCs are blocked by the sieving effect of the membrane pore size and/or adhered on the membrane.

W-3065

MAPK ACTIVITY MAY MEDIATE THE DEGREE OF ADIPOSE-DERIVED STEM CELL OSTEOGENESIS

Patricia Zuk[1], Eric J. Tsang[2], Benjamin Wu[3]
[A]Surgery, [B]Biomedical Engineering, [C]Department of Biomedical Engineering, [1]University of California, Los Angeles, CA, United States

Numerous studies have identified a critical role for MAPK signaling in mediating osteogenic differentiation. To assess this signaling cascade's role in Adipose-derived Stem Cell (ASC) osteogenesis, MAPK signaling was functionally assessed in multiple ASC populations induced toward the osteogenic lineage using several inductive conditions. Increased ASC mineralization with respect to non-induced controls was measured upon induction with ascorbic acid and β-glycerophosphate alone, with levels increasing further by adding dexamethasone or 1,25-dihydroxyvitamin D3 (VD3). While heterogeneity in ASC mineralization was found, mineralization capacity appeared to correlate to kinase activity. Specifically, increased mineralization upon induction with dexamethasone (Dex) coincided with significant increases in ERK1/2 or JNK1/2 activation (i.e. pERK, pJNK) with respect to controls. A synergistic effect was noted as ASCs with the highest mineralization capacities also showed increases in both pERK and pJNK. No discernible relationship between mineralization and kinase activation was observed using hydrocortisone rather then Dex. However, VD3 induction gave similar results to Dex, suggesting that these osteogenic agents may act through downstream MAPK pathways. Consistent with this, increased mineralization levels could be induced using recombinant wild-type lentiviruses designed to overexpress either ERK1 or ERK2 (i.e. ERK1wt, ERK2wt). In contrast, ASC mineralization capacity could be inhibited using pharmacologic inhibitors to MAPK kinase activation. Inhibitor administration at the start of osteogenic differentiation significantly inhibited AP activity and mineralization, suggesting a role for MAPK activity in ASC osteogenic commitment. Mineralization capacity was also decreased using these inhibitors prior to the onset of mineralization or throughout osteogenesis, suggesting additional roles for MAPK signaling in ASC osteogenesis. Transduction of osteo-induced ASCs with ERK1/2 or JNK1/2 shRNA lentiviruses also decreased mineralization. In support of a role for MAPK signaling in ASC osteogenesis, enhanced mineralization capacity and increased kinase activation were also associated with decreased MAPK phosphatase expression. Inhibition of phos-

phatase activity not only reduced kinase activation to basal levels but significantly reduced mineralization capacity. Finally, the effects of MAPK signaling on ASC osteogenesis was assessed in vitro using populations transduced with ERK1wt and ERk2wt lentiviruses and implanted into critical-sized parietal defects in athymic rats. In summary, the results indicate that the degree of ASC osteogenesis may be related to the level of ERK and JNK activation and may involve a complex interplay between kinase and phosphatase activity.

W-3066

ISOLATION AND CHARACTERIZATION OF MESENCHYMAL STEM CELLS FROM BOVINE AMNIOTIC FLUID AT DIFFERENT GESTATIONAL AGES

Barbara Rossi[1], Barbara Merlo[1], Eleonora Iacono[1], Pier Luigi Tazzari[2], Francesca Ricci[2], Cesare Galli[3]
[1]Department of Veterinary Medical Sciences, University of Bologna, Ozzano Emila (BO), Italy, [2]Service of Transfusion Medicine, S.Orsola-Malpighi Hospital, Bologna, Italy, [3]Avantea, Laboratory of Reprodutive Technologies, Cremona, Italy

Introduction - Amniotic fluid (AF) is a reservoir of multipotent mesenchymal stem cells (MSCs), very promising cells for tissue engineering in clinical application. The aim of this work was to isolate and characterize bovine MSCs from AF (bAF-MSCs) as alternative sources of primitive multipotent stem cells in a species that could be an attractive large-animal model for biomedical and biotechnology researches. Materials and Methods - Samples were recovered, at slaughterhouse, from 18 pregnant cows at different gestational ages established on crown-rump length (1: 0-105 d; 2: 106-160 d; 3: 161-200 d; 4: 201-240 d; 5: 241 d-term of pregnancy). Cells were isolated by centrifugation and cultured in DMEM-TCM 199 (1:1) plus 10% FBS. At passage (P) 4, chondrogenic, osteogenic and adipogenic differentiation were induced (n=16) and evaluated morphologically and cytologically (Alcian Blue, Von Kossa and Oil Red O stainings respectively). Phenotypic characterization for stem cell markers was performed at P3 or P4 by flow cytometry for CD105, CD90, CD45 and CD14 (n=16), by immunocytochemistry (ICC) for Oct4, SSEA1, SSEA4 and α-SMA (n=4) and by RT-PCR analysis for Oct4, Nanog and Sox2 (n=7). In both ICC and RT-PCR, bovine fibroblasts were used as negative control. Results - Mean collected AF volume was 35 ml and 200-3636 cells/ml were recovered. Cells were isolated from 16/18 samples. At P0, cell populations appeared heterogeneous and we could identify 4 different cell types: round, spindle-shaped, fibroblastoid and large-flat cells. These different populations were observed at every gestational age analyzed. Through passages, only round cells (n=15/16) predominated. In only one sample, spindle-shaped cells prevailed. After 30 d of culture mean cell doublings were 14,1±3,2 and mean doubling time was 57,7±27,9 hrs without differences related to gestational age or sample ($p>0.05$). Cells were able to differentiate into osteocytes and adipocytes; chondrogenesis was clearly obtained only from samples recovered within 140 d of pregnancy (n=9). Percentage of cells positive for CD90 was 73,0±17,2, for CD105 45,8±35,7, for CD14 5,6±11,2 and for CD45 13,4±11,3; no differences were observed among different gestational ages ($p>0,05$). ICC demonstrated low presence of SSEA4 positive cells (0,05%) in 1 sample, the presence of α-SMA in all 4 samples (mean 11,8±14.1%, range 2.5-32.4%) and the lack of Oct4 and SSEA1. RT-PCR analysis, performed at P4, showed expression of Nanog in 1/7 sample and weak expression in 4/7 samples. Low expression of SOX2 was detected in 2/7 samples. Conclusions - Based on these preliminary results, in the bovine AF there is an heterogeneous cell population containing also multipotent

MSCs, identified by their differentiation ability and phenotypic characteristics typical of MSCs. Unlike human AF-MSCs, only a fraction of bAF-MSCs are positive for some pluripotency markers. Current work is ongoing to further characterize other cell phenotypes selected after long term culture.

W-3067

QUANTIFICATION OF CARTILAGE-SPECIFIC GENE EXPRESSION IN CHONDROGENIC CULTURES OF HUMAN AMNIOTIC FLUID AND MARROW MSCS IN COMPARISON WITH CHONDROCYTES PRESENTED IN HYALINE CARTILAGE

Fatemeh Safari[1], Mohamad reza Baghaban Eslaminejad[2]

[1]*Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran, Islamic Republic of,* [2]*Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran, Islamic Republic of*

Introduction: While **c**artilage diseases result in chronic pain and decreased quality of life, cartilage tissue possess a limited capacity to heal. Cell therapy approaches using bone marrow-derived mesenchymal stem cells (MSCs) represent a novel strategy in dealing with treatment of cartilage diseases. Unfortunately, bone marrow tissue as a source of MSCs exhibits some drawbacks due to its invasive and painful obtaining procedure. To address this problem, scientists have long been searching for providing an alternative source, which contains MSCs population. Amniotic fluid, as one of the readily available sources, has drawn many attentions. In the present study, MSCs were isolated from human amniotic fluid and their level of in vitro chondrogenic differentiation were compared to that of marrow-derived MSCs using quantification of expression of some cartilage-specific genes. In this study, differentiation level of both cells was compared to that of native chondrocyte presented at hyaline cartilage tissue.
Methods: Amniotic fluid was collected from patients undergoing cesarean and transferred to the cell culture Lab where the fluid was centrifuged and the cell pellet was plated under standard condition. Bone marrow was obtained from patients who were volunteer for cell therapy at Royan institute. Mononuclear cells were enriched via density gradient centrifugation and then cultured. Passaged-3 cells from both tissues were pelleted under 300g and treated with standard chondrogenic medium for a period of 3 weeks. Normal human hyaline cartilage was obtained from the patients who had car accident and referred to hospital. Cartilage produced from stem cell differentiation as well as the normal cartilage tissue were histologically prepared for toluidine blue staining. Furthermore, total RNA was collected from the chondrogenic cultures as well as the normal hyaline cartilage and quantitative PCR was performed for the cartilage specific genes.
Results: According to our observation, chondrogenic culture from amniotic fluid stained more intensive than that from marrow MSCs. The amount of metachromatic matrix produced at either culture tended to be significantly lower than that exist at normal hyaline cartilage. According to our findings, amniotic fluid stem cells appeared to express cartilage specific genes including *SOX9, COLLAGEN II, AGGRECAN* and *PERLECAN* at expression levels significantly more than their marrow counterparts ($p<0.05$). Although the expression level of amniotic fluid stem cells tended to be lower than that in normal chondrocytes, but they relatively showed more similarity to normal cartilage tissue than marrow MSCs.
Conclusions: Taken together, chondrocytes produced from chondrogenic differentiation of either amniotic fluid stem cells or marrow MSCs possess significantly less cartilage-specific gene expression level compared with those of normal chondrocytes present at hyaline cartilage. In this regards, amniotic fluid-derived chondrocyte is more similar to native cells; hence preferred cells for cell-based treatment of cartilage defects.

W-3068

PLURIPOTENCY AND DIFFERENTIATION OF STEM CELLS CULTURED ON BIOMATERIALS GRAFTED WITH EXTRACELLULAR MATRIX HAVING DIFFERENT ELASTICITY

Chia-Ju Hsu, Yi-Wei Huang, Li-Yu Chen, Pin-yu Wang, Akon Higuchi

Department of chemical and Materials Engineering, National Central University, Jhongli City, Taiwan

Mesenchymal stem cells (MSCs) are a valuable cell source for tissue engineering and regenerative medicine. Stem cells derived from amniotic fluid (AFSCs) are pluripotent fetal cells capable of differentiating into multiple lineages, containing representatives of all the three embryonic germ layers. AFSCs are categorized as the intermediate stage between the embryonic stem cells (ESCs) and adult stem cells, and AFSCs may have a distinct mechanism to choose their fate. Therefore, amniotic fluid represents a rich and more suitable source of stem cells in regenerative medicine and tissue engineering than ESCs and induced pluripotent stem cells (iPSCs) due to the lack of ethical concerns regarding use of ESCs and the lack of concerns about xenogenic contamination arising from the use of mouse embryonic fibroblasts as a feeder layer for iPSCs and ESCs. However, stem cells fate of decision is regulated not only by stem cells themselves, but also the effect of microenvironment of stem cells. Therefore, stem cell microenvironment surrounding specific matrix proteins should be important to decide the stem cell fate of differentiation. The optimal design of stem cell culture biomaterials will facilitate the in-vitro production of the large numbers of pluripotent stem cells and specifically more numbers of differentiated cells, which are demanded in the regenerative medicine. In this study, we investigated whether the stiffness of cell adhesion substrates affect the maintenance of pluripotency of the stem cells and/or modulated the stem cell fate of differentiation. Stem cells from amniotic fluid were cultured on different stiffness of polyvinylalcohol-co-itaconic acid (PVA-IA) films grafted with extracellular matrix (ECM) dishes where collagen, fibronectin, and vitronectin were selected for further studies as ECM components (nanosegments). PVA-IA films were selected as the base matrix due to easy regulation of elasticity (22 kPa-16.5 MPa) by changing the crosslinking time (15 min - 48 hr). AFSCs on soft substrates (22 kPa-1 MPa) had less spreading, fewer stress fibers and lower proliferation rate than AFSCs on stiff substrate (3MPa-16.5 MPa). The effects of interaction between AFSCs and ECMs (nanosegments) were investigated on the expression of pluripotent genes (e.g., Nanog, SOX2, and Oct4) of AFSCs and on the differentiation abilities of AFSCs into several lineage, which were cultured on PVA-IA films grafted with collagen, fibronectin, and vitronectin having different elasticity at passage 4. It was found that AFSCs on PVA-IA films grafted with ECM of elastic modulus (500 kPa-800 kPa) in the intermediate showed higher pluripotency genes than those on soft or hard substrates. AFSCs could differentiate into neural (nestin), smooth muscle cell (α-actin), or osteogenic (Runx2) phenotypes depending on whether they were cultured on PVA-IA films grafted with ECM substrates of elastic modulus in the lower, intermediate or higher ranges. AFSCs cultured on soft substrates
have weaker cell adhesion, and showed higher expression of early marker of neural cells (nestin) in expansion medium without addition of induction molecules.

W-3071

INTERIM ANALYSIS OF 12 ALS PATIENTS TREATED WITH DIFFERENTIATED MESENCHYMAL STEM CELLS IN A PHASE I/II CLINICAL TRIAL SHOWS SAFETY AND SOME INDICATIONS OF CLINICAL BENEFIT

Daniel Offen[1], Panayiota Petrou[2], Marc Gotkine[2], Yael Gothelf[1], Yossef Levy[1], Adi Vaknin-Dembinsky[2], Zohar Argov[2], Tamir Ben-Hur[2], Eldad Melamed[1], Dimitry Karusiss[2]
[1]*Brainstorm Cell Therapeutics Ltd, Peta-Tikva, Israel,* [2]*Department of Neurology, Hadassah Hebrew University Medical Center, Jerusalem, Israel*

Background: Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's disease, is a fatal, rapidly progressive neurodegenerative disease caused by motor neuron degeneration. Despite extensive efforts, no currently available treatment has been shown to slow down, halt, or reverse disease progression. Bone marrow-derived mesenchymal stem cells (MSC) represent an attractive cell source for the treatment of neurological diseases, with the potential to exert neuroprotection and possibly induce neurogenesis. We have previously demonstrated the neuroprotective effects of BrainStorm's NurOwn™ cells (Autologous Neurotrophic Factor (NTF)-secreting MSC, or "MSC-NTF") in various animal models of neurodegenerative diseases, including ALS. In the current study, we demonstrated the clinical safety and initial indications of clinical benefit of NurOwn cells in twelve ALS patients.

Aims: To evaluate the safety and tolerability of treatment with autologous MSC-NTF in ALS patients utilizing intramuscular (IM) injection of the cells in the early stage of the disease and intrathecal (IT) injection in patients with more advanced/progressive disease.
Methods: Bone marrow-derived MSC were isolated from twelve ALS patients, expanded ex-vivo and induced to secrete NTF using Brainstorm's NurOwn™ differentiation technology. These cells were transplanted via IM (at 24 sites: 2x105 cells per site) or IT (1x106 cells/kg) injections to patients with early (ALSFRS score of >30; n=6) or advanced ALS (ALSFRS: 15-30; n=6), respectively. Patients were monitored clinically on a monthly basis for 3 months pre-treatment and 6 months post-transplantation.
Results: During the six-month follow-up of the transplanted patients, no serious treatment-related adverse events were observed, indicating short-term treatment safety. The clinical follow-up of the patients also revealed initial indications of beneficial clinical effects in some of the MSC-NTF transplanted patients, as evidenced by a significant change in the rate of clinical progression (ALSFRS), respiratory function, electrophysiology and 3D-MRI volumetric evaluation of the muscles, as compared to the 3 months preceding the treatment.
Conclusions: Preliminary results of our Phase I/II ALS trial with autologous IM/IT transplantation of MSC-NTF indicate that single treatment at the current dosage appears to be safe. The initial indications of beneficial clinical effects may pave the way for future larger trials. Further analysis of the data and longer term follow-up of the participating patients are needed to confirm these observations.
Trial registration: ClinicalTrials.gov (ClinicalTrials.gov Identifier: NCT01051882). The study is sponsored by Brainstorm Cell Therapeutics Ltd.

W-3072

APOLIPOPROTEIN A-I PROMOTES OSTEOGENESIS THROUGH STAT3 ACTIVATION AND AMELIORATES THE OVARIECTOMY-INDUCED BONE LOSS

Yu-chuan Liu
Genomic Research Center, Academia Sinica, Taipei, Taiwan

Apolipoprotein A-I (ApoA-I) is a major component of human high-density lipoproteins (HDLs) that remove cholesterol from arteries. The decrease of ApoA-I express levels increases the chances of cardiovascular disease, obesity, or other metabolic syndrome. By a high throughput screen, we identified ApoA-I can promote activity of early and late osteogenesis markers alkaline phosphatase (ALP) (6-8 fold) and Alizarin red staining (40 fold) in mesenchymal stem cells (MSCs). Furthermore, ApoA-I increased the expression of osteogenic markers RNA of ALP (18 fold) and bone sialoprotein (BSP) (6 fold). In addition, the phosphorylation of signal transducer and activator of transcription 3 (STAT3) and extracellular signal-regulated kinases (ERK) are both increased upon ApoA1 treatment. Inhibition of STAT3 activity or ERK activity by inhibitor diminished the effect of ApoA-I in osteogenic differentiation. ERK inhibitor also blocks STAT3 phosphorylation. Thus ERK may be the upstream regulator of STAT3. Meanwhile, we identified many cytokines stimulated by ApoA-I are regulated by STAT3. The knockdown of these cytokines blocks the osteogenesis of MSCs. The functions of these cytokines were not reported in ApoA-I-mediated pathways or bone formation before. In vivo, we further demonstrate that ApoA-I transgenic mice can prevent the osteoporosis in ovariectomized mice, a model mimic osteoporosis occurs in postmenopausal women. Osteoporosis is an important disease that indirectly leads to more death in peoples than breast cancer. One-third of the women and one-fifth of the men experience of osteoporotic fracture and the one-year mortality rate of hip fracture is 28-35%. Taken together, we find an ApoA-I-ERK-STAT3 pathway can promote osteogenic differentiation in vitro, and ApoA1 can prevent the bone loss in ovariectomized mice. This study not only identified a new ApoA-I-mediated pathways in bone formation, it may also provide several potential therapeutic targets for bone disease like osteoporosis or bone fracture.

W-3073

BASIC FIBROBLAST GROWTH FACTOR- INDUCED FUNCTIONAL NEURONAL DIFFERENTIATION OF CANINE BONE MARROW STROMAL CELLS

Rei Nakano, Kazuya Edamura, Kenji Teshima, Kazushi Asano, Tomohiro Nakayama, Takanori Narita, Ken Okabayashi, Hiroshi Sugiya
Nihon University, Fujisawa, Japan

In the veterinary medicine, the clinical trial of spinal regenerative therapy using autologous bone marrow stromal cells (BMSCs) has been performed in dogs. However, the capacity of neuronal differentiation of canine BMSCs has not been well elucidated, although which is very important for the clinical application of canine BMSCs. In the previous studies, most of canine BMSCs died during or after the differentiation and it was obscure whether the differentiated cells had neuronal functions. In this study, we demonstrate that basic fibroblast growth factor (bFGF) is effective for differentiation of canine BMSCs into functional neurons.
Canine bone marrow was aspirated from the humerus of healthy beagle dogs. This study was conducted under Nihon University Animal Care and Use Committee approval. Mononuclear cells were separated by density gradient centrifugation using Histopaque-1077 (Sigma-Aldrich Inc.). The mononuclear cells were then static-cultured using α-MEM with 10% fetal bovine serum. On the fourth day of culture, non-adherent cells were removed when the culture medium was replaced, thus isolating the canine BMSCs. For neuronal induction, the canine BMSCs were incubated in Neurobasal-A medium (Life Technologies Co.) supplemented with 2% B27 supplement (Life Technologies Co.) containing bFGF (Immunostep: 100 ng/mL). Viability was assessed by trypan-blue exclusion. Real-time RT-PCR was performed to evaluate the expression of mRNAs of neural stem cell (NES), neuron (MAP2, NEFL, ENO2) and glia (GFAP) markers. To examine the cellular localization of neuronal markers, such as neurofilament light chain (NF-L) and neuron-specific enolase (NSE), immunofluorescence confocal microscopy was performed. To investigate whether the neuron-like cells from canine BMSCs were functional, we determined Ca^{2+}mobilization by using the Ca^{2+} indicator fluo-3.
When canine BMSCs were incubated with bFGF, viability of the cells was maintained up to 10 days. In the bFGF-treated cells, the mRNA expression of neuron markers such as MAP2, NEFL and ENO2 increased, while that of the neural stem cell marker NES and the glia marker GFAP decreased. The MAP2 mRNA expression induced by bFGF was completely inhibited by the FGFR inhibitor SU5402 (25 μM). The bFGF-treated cells exhibited round cell bodies and had several long, sharp processes like neurons. The expression of the neuron marker NF-L and NSE was confirmed in the bFGF-treated cells by immunocytochemistry. In the fluo 3-loaded bFGF-treated cells, KCl (50 mM) and $_L$-glutamate (100 μM) elicited an increase in intracellular Ca^{2+} levels, suggesting that voltage-dependent Ca^{2+} channels and glutamate receptors function in the cells.
Taken together, it is most likely that bFGF induces differentiation of canine BMSCs into functional neurons.

W-3074

BONE MORPHOGENIC PROTEIN-2 SHOWS DIFFERENTIAL ACTIONS ON HUMAN ADIPOSE TISSUE AND BOME MARROW DERIVED MESENCHYMAL STEM CELLS

Sun Young Lee[1], Jee Young Kim[1], Da sol Kim[1], Yong Chan Bae[2], Keun Tak Suh[3], Jin Sup Jung[1]
[A]Physiology, [B]Plastic Surgery, [C]Orthopaedic Surgery, [1]School of Medicine, Pusan National University, Yangsan, Korea, Republic of

The bone morphogenetic proteins (BMPs) belong to a unique group of proteins that includes the growth factor TGF-β. BMPs play important roles in cell differentiation, cell proliferation, and inhibition of cell growth. BMPs can induce the differentiation of mesenchymal progenitor cells into various cell types, including chondroblasts and osteoblasts. The aim of this study was to analyze the effect of BMP-2 on the adipogenic and osteogenic differentiation of human adipose tissue- (hADSC) and bone marrow (hBMSC)-derived mesenchymal stem cells.BMP-2 increased osteogenic differentiation in human bone marrow-derived MSCs (hBMSC) without affect their adipogenic differentiation. Unexpectedly, BMP-2 increased adipogenic differentiation of hADSC without affecting osteogenic differentiation at an osteogenic differentiation condition. BMP-2 did not enhance adipogenic differentiation at an adipogenic differentiation condition. Real time PCR analysis showed that hADSC express BMP receptors and SMAD1 and 4, and that BMP-2 increased the expression of BMP2-responsive genes and induced SMAD1 phosphorylation in hADSC.

Proteome analysis showed that BMP2 increased adipogenesis-related protein levels. Downregulation of SMAD1 and 4 by the specific siRNAs transfection inhibited BMP2-induced increase of adipogenic differentiation in hADSC. At the condition that adipogenic differentiation was inhibited by the treatment of TNF-α, BMP2 stimulated osteogenic differentiation of hADSC. These data indicated that the control of osteogenesis and adipogenesis in MSCs are closely related, and that hADSC have preferential commitment into adipogenic lineages.

W-3075

PANCREATIC EXTRACT PROMOTES BONE MARROW DERIVED MESENCHYMAL STEM CELLS DIFFERENTIATION INTO INSULIN PRODUCING CELLS

Hongbin Xie, Yunshuai Wang, Hui Qi, Hanxie Zhou, **Fu-Rong Li**
Laboratory of stem cell and Cellular therapy, The Second Clinical Medical College,Jinan University, Shenzhen, China

Mesenchymal stem cells can be successfully induced to differentiate into insulin-producing cells by a variety of small molecules and cytokines *in vitro*. However, problems remain, such as low transdifferentiation efficiency and poor maturity of transdifferentiated cells. The damaged pancreatic cells secreted a large amount of soluble proteins, which were able to promote pancreative islet regeneration and mesenchymal stem cells differentiation. In this study, we utilized the damaged pancreatic tissue extract to modulate mesenchymal stem cells differentiation into insulin-producing cells by the traditional two-step induction. Our results showed that damaged pancreatic tissue extract could effectively improve the transdifferentiation efficiency and maturity of insulin-producing cells by the traditional induction method.Our study provides a new strategy to induce an efficient and directional differentiation of mesenchymal stem cells into insulin-producing cells.

W-3076

IN VITRO DIFFERENTIATION OF BONE MARROW DERIVED RAT MESENCHYMAL STEM CELLS TO NEURON-LIKE CELLS USING SPECIFICALLY DESIGNED NICHE

INDU S, TARA S
Thrombosis Research Unit, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram, India

Bone marrow mesenchymal stem Cells (BMMSCs) are adult multipotent stem cells which give rise to hematopoietic and non hematopoietic lineage cells *in-vivo*. Reports also indicate the differentiation of these BMMSCs into many cell types including cardiac, hepatic lineages. Even though, BMMSC differentiation into neuron-like cells (NLC) is reported, controversies exist regarding whether these cells truly represent neurons or not. The transplantation of neurons into injured/ degenerated sites is not effective in regenerative therapy as neurons are terminally differentiated cells. Since MSCs are multipotent, transplantation of these cells may not effective for achieving desired cell type at the site of injury. So precursors of neuron should be more appropriate for transplantation in regenerative medicine. This study hypothesizes that a cell-specific composition of bio mimetic matrix and culture medium together can support bone marrow derived neural progenitor cells (NPCs) for longer time in culture. The objective of the study was to identify the niche composition that can induce neural lineage commitment of BMMSC and support long term survival without phenotype drift.
The isolation and *in-vitro* culture of BMMSC from rat (Wistar) was done with IAEC approval. Briefly, Wistar rats (two month old) were sacrificed by CO_2

inhalation and the tibias and femurs were dissected out under sterile conditions. The bones were placed in HBSS buffer and were cleared of any muscle tissue using a sterile scalpel. DMEM F-12 medium containing 10% FBS was passed through the cut ends of the bone using a 18 G syringe and the marrow plugs were flushed out. The single cells obtained after re suspension were centrifuged at 400*g* for 5 min at 37°C. The bone marrow cells were seeded at a density of 25 x10^6 cells/ml DMEM F-12/25 cm^2 bare polystyrene surface (PS). For differentiation experiment, isolated, culture-expanded and characterized MSCs were plated on to the culture matrix. Matrix was prepared by clotting thin layer of fibrinogen composite, comprising fibronectin, hyaluronic acid, hypothalamus extract (HE) and platelet growth factors (PGF), on thrombin adsorbed PS. Differentiation was attempted using two media compositions viz; basic DMEM-F12-FBS containing (i) basic fibroblast growth factor (b FGF) [medium I] and (ii) HE and PGF [medium II]. Characterization of NLCs after defined periods of MSCs culture was done using immunocytochemistry. Homogeneity of BMMSCs was confirmed using positive markers; $CD90^+$, $CD44^+$, c-kit^+, $CD\ 105^+$ and negative marker $CD45^-$ using flow cytometry. Morphologically, healthy NLCs were observed in fibrin coated dishes in comparison to those found in uncoated dishes. The NLCs were positive for neuron specific markers like β-tubulin, MAP2 and synaptophysin at different stages of culture.. Media I and II supported the growth of NLCs; however enhanced NLC density was found in dishes containing medium II. The introduction of 2 mM Valproic Acid (VA) into medium II increased axonal elongation. NLC morphology showed minor changes upon prolonged culture. Long term maintenance of NLCs without any morphological changes was achieved by supplementing medium II with 2mM VA and minimal levels (2ng/mL) of FGF. **Conclusion:** Adhesive matrix and medium composition together created a cell specific niche that induced BMMSCs differentiation into NLCs and specific medium compositions supported their prolonged survival.

W-3077

DEVELOPMENT OF A 3D COLLAGEN GEL VASCULARIZED TISSUE-ENGINEERED CONSTRUCT FOR BONE REPAIR AND REGENERATION USING EMBRYONIC AND POSTNATAL PROGENITORS

Mani T. Valarmathi[1], Biechler V. Stefanie[2]

[1]Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL, United States, [2]Cell Biology and Anatomy, University of South Carolina, Columbia, SC, United States

One of the major challenges in engineering complex tissues such as bone, which involves the reconstruction of 3D shapes and internal architectures, is the need to vascularize the tissue in vitro for functionally competent vascular networks. Vascularization in vitro could maintain cell viability during tissue growth, induce structural organization and promote neovascularization upon implantation. A method to enhance graft vascularization is to establish a primitive vascular plexus within the implant before transplantation by the use of cellular-based concepts. Consequently, this necessitates the formation of appropriate in vitro 3-D plexuses of new blood vessels within the pre-implanted biomaterial constructs through the process of in situ de novo vasculogenesis/angiogenesis for organ tissue engineering. It is well established that the process of neovascularization or neoangiogenesis is coupled to the development and maturation of bone. Bone marrow stromal cells (BMSCs) or mesenchymal stem cells (MSCs)

comprise a heterogeneous population of cells that can be differentiated in vitro into both mesenchymal and non-mesenchymal cell lineages. When both rat BMSCs and quail proepicardia (PEs) were seeded onto a three-dimensional (3-D) tubular scaffold engineered from aligned collagen type I strands and co-cultured in osteogenic media, the maturation and co-differentiation into osteoblastic and vascular cell lineages were observed. In addition, these cells produced abundant mineralized extracellular matrix materials and vessel-like structures. BMSCs were seeded at a density of 2 × 106 cells/15 mm tube and cultured in basal media for 3 days. Subsequently, on day 3, PEs were seeded onto the same tubes and the co-culture was continued for another 3, 6 or 9 days either in basal or in osteogenic media. Differentiated cells were subjected to immunohistochemical, cytochemical and biochemical analyses. Phenotypic induction was analyzed at mRNA level by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). Immunolocalization of key osteogenic and vasculogenic lineage specific markers were examined using confocal scanning laser microscopy. In osteogenic tube cultures, both early and late osteogenic markers were observed and were reminiscent of in vivo expression pattern. Alkaline phosphatase activity and calcium content significantly increased over the observed period of time in osteogenic medium. Abundant interlacing fascicles of QCPN, QH1, isolectin and α-smooth muscle actin (α-SMA) positive cells were observed in these tube cultures. These cells formed extensive arborizations of nascent capillary-like structures and were seen amidst the developing osteoblasts in osteogenic cultures. The 3D culture system not only generated de novo vessel-like structures but also augmented the maturation and differentiation of BMSCs into osteoblasts. Thus, this novel co-culture system provides a useful in vitro model to investigate the functional role and effects of neovascularization in the proliferation, differentiation and maturation of BMSC derived osteoblasts. We therefore demonstrate that under appropriate in vitro physicochemical microenvironmental cues (combination of growth facts and ECM) multipotent adult BMSCs in combination with embryonic-derived progenitors, on 3D collagen gel based scaffolds engineered from aligned collagen type I fibers in various media conditions to generate numerous vascularized tissues.

W-3078

ENGAGING NATIVE CELLS WITH BIOINSPIRED MATRICES

Shyni Varghese, **Shih Yuru Vernon**, Yamaguchi Tomonori, Ameya Phadke, Masuda Koichi
Univ of California, San Diego, La Jolla, CA, United States

Advancements in stem cell biology and engineering offer unprecedented therapy for treating compromised tissues and cells. However, the challenge remains of how to effectively harness the therapeutic potential of stem cells to repair the targeted tissue/cell in vivo with control and precision. One of the widely recognized approaches involves transplantation of stem cells with the aid of delivery vehicles such as biomaterials, which provide structural support and instructive cues. Though biomaterials-assisted transplantation of cells is a promising approach to repair compromised tissues, developing systems that can activate endogenous stem cells and contribute to tissue regeneration are more clinically attractive. By employing a biomineralization approach, we have recently created a synthetic matrix emulating various functional attributes of native bone tissue. Human mesenchymal stem cells (hMSCs) and human embryonic stem cells (hESCs) cultured on these mineralized matrices underwent osteogenic differentiation in the absence of any osteogenic inducing soluble factors. Interestingly, when implanted in vivo, the acellular matrices recruited endogenous cells and formed vascularized bone tissue. Additionally, the acellular mineralized matrices also enabled fusion of decorticated transverse processes in a posterolateral lumbar fusion model. Radiographic and mechanical lateral bending analyses revealed that both cellular and acellular approaches promoted spinal fusion to a similar extent. Furthermore, we have employed these engineered matrices as an “artificial extracellular matrix” to understand the mechanism by which the calcium phosphate minerals induce osteogenic differentiation commitment of stem cells and the role of minerals on bone physiology. Perturbation of SLC201, a trans-membrane phosphate transporter, abrogates the matrix-induced osteogenic differentiation of hMSCs by decreasing intra-mitochondrial phosphate content and ATP synthesis. Moreover, adenosine, an extracellular ATP metabolite, was found to act as an autocrine and paracrine signaling molecule for osteogenic commitment of stem cells through A2b adenosine receptor. These findings begin to shed light on the role of ATP metabolism on bone homeostasis, which may be exploited to treat bone metabolic diseases. Furthermore, such biomimetic matrices that can activate endogenous cells and can control their fate to create functional tissues could have a significant

impact on regenerative medicine. Such synthetic matrix assisted tissue regeneration offers an easy-to-use therapeutic strategy to treat critical bone defects.

W-3081

ACELLULAR CROSS-LINKED BOVINE PERICARDIUM COATED WITH NANOFIBERS FOR TISSUE ENGINEERING

Santosh Sadashiv Mathapati[1], Dillip Kumar Bishi[1], Jayarama Reddy Venugopal[2], Kotturathu Mammen Cherian[1], Soma Guhathakurta[1], Seeram Ramakrishna[3], Rama Shanker Verma[4]

[1]Stem cell and Tissue engineering, Frontier Life Line Pvt Ltd, Chennai, Chennai, India, [A]Nanoscience and Nanotechnology Initiative, Faculty of Engineering,, [B]Nanoscience and Nanotechnology Initiative, Faculty of Engineering, [2]National University of Singapore, Singapore, Singapore, [3]Stem Cell and Molecular Biology Laboratory, Department of Biotechnology, Indian Institute of Technology Madras, Chennai, India

Aim: Bovine pericardial tissues are widely used for the construction of a variety of bioprostheses. They are used to repair complex anatomical tissue defects arising out of cardiac and non-cardiac conditions such as soft tissue repair, abdominal wall defect, and strengthening the suture line during general surgical procedures. The present study is aimed at improving nanofiber coating of acellular tissue-engineered bovine pericardium (ATEBP) with biological polymers to nurture cells for tissue morphogenesis, improve adhesion and mechanical properties with the objective of directing cells for self-conclave in three dimensions. Such constructs confer exquisite functionality such as human mesenchymal stem cell (hMSCs) differentiation into endothelial cells (ECs) for tissue engineering.
Methods: Nanofibers of poly(L-lactic acid)-co-poly(ε-caprolactone) (PLACL) and also blend of PLACL/gelatin (PLACL/gelatin) were coated on with earlier developed acellular glutaraldehyde cross-linked detoxified bovine pericardium (ATEBP) scaffolds. Fabricated electrospun nanofibrous scaffolds were analyzed for fiber diameter, functional groups, hydrophilic or hydrophobic nature and mechanical properties. ATEBP coated with nanofibrous scaffolds were assessed for the proliferation of hMSCs and their differentiation into ECs.
Results: The ATEBP scaffolds coated with PLACL/gelatin nanofibres exhibited an increase in hydrophilicity and decrease in average fiber diameter and mechanical properties. The high porosity of PLACL/gelatin at above 70% is beneficial for the adhesion and proliferation of the hMSCs in addition to facilitating a homogeneous three-dimensional distribution of cells. Moreover, the increased gelatin content decreases fiber diameter, which leads to decreased porosity of scaffolds and biomechanical properties but enhanced cell adhesion, proliferation, and differentiation. The results showed that increased hMSCs adhesion, proliferation, and differentiation into ECs on ATEBP coated with PLACL/gelatin nanofibrous scaffolds compared to PLACL-coated ATEBP.
Conclusion: The ATEBP-coated nanofibrous scaffolds of PLACL/gelatin pave way for better hMSCs adhesion, proliferation, and differentiation into ECs to improve the therapeutic value of current soft tissue constructs in tissue engineering.

W-3082

INTRACAVERNOUS DELIVERY OF CLONAL MESENCHYMAL STEM CELLS RESTORES ERECTILE FUNCTION IN A MOUSE MODEL OF CAVERNOUS NERVE INJURY

Ji-Kan Ryu[1], Kang Moon Song[1], TacGhee Yi[2], Jun-Kyu Suh[1], **Sun U. Song**[2]

[A]Department of Urology, [B]Translational Research Center, [1]Inha University School of Medicine, Incheon, Korea, Republic of

Recently, much attention has focused on stem cell therapy; bone marrow-derived stem cells (BMSCs) are one of the most studied mesenchymal stem cells used in the field of erectile dysfunction (ED). However, a major limitation for the clinical application of stem cell therapy is the heterogeneous nature of the isolated cells, which may cause different treatment outcomes. We investigated the effectiveness of mouse clonal BMSCs obtained from a single colony by using subfractionation culturing method (SCM) for erectile function in a mouse model of cavernous nerve injury (CNI). Twelve-week-old C57BL/6J mice were divided into 4 groups: sham operation group, bilateral CNI group

receiving a single intracavernous (IC) injection of phosphate-buffered saline (PBS, 20 μL) or clonal BMSCs (3×10^5 cells/20 μL), and bilateral CNI group receiving a single intraperitoneal (IP) injection of clonal BMSCs (3×10^5 cells/20 μL). The clonal BMSC line was analyzed for cell-surface epitopes by using fluorescence-activated cell sorting and for differentiation potential by using *in vitro* differentiation assays. Two weeks after bilateral cavernous nerve crushing and treatment, erectile function was measured by electrically stimulating the cavernous nerve. The penis was harvested for histologic examinations and western blot analysis. Clonal BMSCs expressed cell surface markers for mesenchymal stem cells and were capable of differentiating into several lineages, including adipogenic, osteogenic, and chondrogenic cells. Both IC and IP injections of clonal BMSCs significantly restored cavernous endothelial and smooth muscle content in CNI mice. IC injection of clonal BMSCs induced significant recovery of erectile function, which reached 90-100% of the sham control values, whereas IP injection of clonal BMSCs partially restored erectile function. We established a homogeneous population of mouse clonal BMSCs using SCM; clonal BMSCs successfully restored erectile function in CNI mice. The homogeneous nature of clonal mesenchymal stem cells may allow their clinical applications.

W-3083

MESENCHYMAL STROMAL CELL DIFFERENTIATION TO ENDOTHELIAL CELLS: ROLE OF NOTCH SIGNALLING.

Samantha M. Roberts, Stephen G. Ball, Cay M. Kielty
Wellcome Trust Centre for Cell Matrix Research, University of Manchester, Manchester, United Kingdom

Notch signalling has been implicated in cell fate decisions and differentiation during vascular development, especially in branching angiogenesis and in the determination of arterial and venous fate. Previous investigations have also identified a role for Notch signalling in mesenchymal stromal/stem cell (MSC) differentiation towards an endothelial lineage (Whyte et al 2010). We are therefore investigating whether Notch signalling supports the endothelial differentiation of MSCs. MSCs seeded onto gelatine-coated 2D wells at high density demonstrated an increase in protein expression of the Notch 1 receptor intracellular domain and mRNA levels of the Hes and Hey transcription factors compared to low density MSCs. In addition a significant increase in the mRNA expression of the endothelial markers, PECAM-1, Tie2, VE-cadherin, von Willebrand factor and Vascular endothelial growth factor receptor 2, was observed after 96 hours in culture. Further increases in these endothelial markers were observed when MSCs were allowed to form spheroid bodies in which Notch signalling is highly active. Within the spheroids, the MSCs exhibited significantly higher levels of PECAM-1, Tie2 and Vascular endothelial growth factor receptor 2 protein expression after 96 hours in culture compared to the high density adherent cells. The effect of Notch signalling inhibition was assessed using the γ-secretase inhibitor DAPT, which resulted in a significant decrease in endothelial marker expression in MSCs cultured in both 2D and 3D high density culture models. Thus the observed increases in endothelial markers in these culture conditions are Notch-mediated. Ongoing studies are further investigating the mechanisms involved and the extent of endothelial commitment. In the future, we hope to modulate Notch signalling in order to differentiate MSCs down an endothelial cell lineage, for cell-based tissue regeneration therapies.

W-3084

SHEAR STRESS INDUCES ENDOTHELIAL LIKE PHENOTYPES IN PORCINE ASCS BUT FAILS TO INDUCE ENDOTHELIAL SURFACE MARKERS

Rafael Dariolli, Vinicius Bassaneze, Gabriela Venturini, Fernando Giugni, Jose E. Krieger
Laboratory of Genetics and Molecular Cardiology, Heart Institute - InCor - University of Sao Paulo Medical School, Sao Paulo, Brazil

Introduction: Adipose tissue-derived cells (ASC's) may have therapeutic applications in cardiovascular cell-based therapies despite the fact that they lack robust trandifferentiation potential. It is believed that the complex interplay between the implanted cells and the injured cardiac microenvironment may be essential for the understanding of their role in therapeutic cardiac protection. Aim: To evaluate the role of shear-stress (SS), one of the phys-

ical forces important for maintenance of cardiovascular homeostasis, on pASCs in vitro. Methods: The pASC immunophenotype was analyzed by flow cytometry. SS experiments were performed using the well-established cone-plate viscometer system. Nitric Oxide (NO) production was assessed by the amount of nitrate and nitrite accumulation in the media of sheared and static cells using the colorimetric Griess assay. The cytokine VEGF-A was measured by ELISA assay. Results: pASC exposed to SS displayed an uncommon LiCl-reversible alignment and GSK-3β phosphorylation after 24 hours, but failed to express endothelial cell (EC) markers (CD31, VE-cadherin, and FLK-1). The SS stimuli resulted in nitric oxide (NO)-induced VEGF accumulation (15dyn/cm², up to 96 hours). Interestingly, SS-induced phosphorylation of ERK and AKT and the release of NO were independent of the magnitude of the stimulus (1-3, 5, 10, 15 and 25-30 dyn/cm², up to 30 minutes). In contrast, long-time (24-48 hours) SS-induced NO and VEGF release in response to 5 dyn/cm² was higher than that in response to 10 or 15 dyn/cm2. Conclusion: Altogether, we provided evidence that the SS induces endothelial-like phenotypes, such as, modulation of NO and VEGF release and morphological alteration despite the fact that pASC failed to express endothelial cell surface markers in vitro. The efficacy of the pre-exposure of transplanted cells for therapeutic purposes to stimuli, such as, SS deserves to be further explored.

W-3085

TRANSMYOCARDIAL REVASCULARIZATION ENHANCES MESENCHYMAL STEM CELL ENGRAFTMENT IN INFARCTED HEARTS THROUGH STEM CELL FACTOR--C-KIT (SCF--C-KIT) AND STROMAL DERIVED FACTOR-1--CHEMOKINE RECEPTOR TYPE 4 (SDF-1--CXCR4) SIGNALING AXES

Uswa Shahzad, Guangming Li, Terrence M. Yau
Division of Cardiovascular Surgery, Peter Munk Cardiac Centre, University Health Network, Toronto, ON, Canada

Transmyocardial Revascularization Enhances Mesenchymal Stem Cell Engraftment in Infarcted Hearts Through SCF–c-kit and SDF-1—CXCR4 Signaling Axes

Objective

We have previously demonstrated that transmyocardial revascularization (TMR) enhances postinfarction myocardial repair by mesenchymal stem cell (MSC) transplantation via signaling to circulating progenitor cells. This was associated with upregulation of components of the stem cell factor (SCF)–c-kit signaling axis and stromal derived factor-1 (SDF-1)–chemokine receptor type 4 (CXCR4) axis. We therefore investigated the roles of the SCF–c-kit and SDF-1–CXCR4 stem cell signaling axes in TMR-augmented repair of infarcted hearts.

Methods

Three weeks after LAD ligation, female Lewis rats underwent 10-channel needle TMR, followed by daily IV injections of 1 million male donor MSCs for 5 days, either wild type (WT) or with knockdown (K/D) of c-kit or CXCR4, accomplished via a shRNA+plasmid in a lentiviral vector. Experimental groups included: WT MSC with or without TMR, c-kit K/D MSC with or without TMR, and CXCR4 K/D MSC with or without TMR (N=6/group). Gene expression was evaluated by quantitative PCR and LV function by echocardiography.

Results

Flow cytometry revealed that *in vitro* cell surface expression of c-kit (N=3) was reduced from 14±0.7% of WT MSCs to 1.6±0.4% in c-kit K/D MSC after one week ($p<0.05$). Similarly, CXCR4 expression (N=3) was reduced from 39±10% of WT MSCs to 3.7±0.7% of CXCR4 K/D MSC at one week ($p<0.05$). Thus, knockdown dramatically reduced receptors key to stem cell signaling.

In our rat infarct model, 3 days after the last MSC injection, the number of MSCs that had homed into the infarct was affected by both TMR and donor cell type, with greater MSC engraftment with TMR and with WT MSC (TMR, cell type, and interaction, $p<0.05$). At 1 week, these differences persisted (TMR and cell type, $p<0.05$).

At 3 days, TMR significantly upregulated transcription of c-kit (TMR, $p<0.05$), SCF (TMR and cell type, $p<0.05$), CXCR4 (TMR and cell type, $p<0.05$), and SDF-1 (TMR and cell type, $p<0.05$). At 1 week, we saw similar declines in expression of c-kit (cell type, $p<0.05$), SCF (TMR, $p<0.05$), CXCR4 (TMR and cell type, $p<0.05$), and SDF-1 (TMR, $p<0.05$).

At 1 week, TMR improved LV ejection fraction (LVEF) (N=5) when WT MSCs were infused, but knockdown of either c-kit or CXCR4 completely abrogated this TMR-mediated augmentation of MSC reparative effect (TMR and cell type, $p<0.05$).

Conclusions

Cell surface expression of c-kit and CXCR4 in MSC is significantly decreased after *ex vivo* knockdown. Downregulation of either c-kit or CXCR4 in MSC decreased homing and engraftment of circulating MSC and inhibited the reparative effects of TMR. Knockdown of either c-kit or CXCR4 completely eliminated TMR-induced improvement of LVEF. Hence, both SCF–c-kit and SDF-1–CXCR4 signaling axes are required for TMR-augmented repair of the infarcted heart. We speculate that variability in stem cell signaling axes and efficacy may also underlie variable clinical responses to TMR in patients.

W-3086

A NOVEL APPROACH FOR THE TREATMENT OF STERILITY USING MESENCHYMAL STEM CELLS ISOLATED FROM MICE'S BONE MARROW

Sherif N. Amin, Hisham H. Issa, Nora A. Khan, Odai Abushalbaq, Walaa Bajaber
general, cell safe bank, cairo, Egypt

Sterility is the failure of sexual active, disabilities of couples to accomplish pregnancy after one year of unprotected intercourse which considered as problem in the society due to its high prevalence rate. Male cause for sterility found in 50% of couples.This research focuses on extracting stem cells from mice bone marrows and expanding them to obtain mesenchymal stem cells that will be then utilized for the treatment of sterility. Mice were first dissected to obtain their femurs and tibiae, which is followed by extraction of cells from bone marrow of these bones. After that, Mesenchymal stem cells were cultured on Mesenchymal stem cell expansion medium and expanded in several subcultures followed by detection and quantitation using flow cytometry in order to be used for treatment of mice infertility. sterile mice were injected with mesenchymal stem cells. after 3 weeks these mice were dissected to study the effect of mesenchymal stem cells on these sterile mice. Results showed that the extracted mesenchymal stem cells from bone marrow showed positive growth and differentiation under specific culture conditions after several subcultures in which these cells were detected and quantified as CD90+ using flow cytometry, which is a main characteristic cell marker of Mesenchymal stem cells. moreover, histochemical results showed minimal spermatogonial cells after dissection of mice. in conclusion mesenchymal stem cells might be of future use in treatment of sterility but with the help of extensive studies in this field.

W-3087

CHARACTERIZATION OF SYNOVIUM-DERIVED CANINE MESENCHYMAL STEM CELLS (MSCS) USING CHEMICALLY DEFINED SERUM FREE MEDIA STK1 AND STK2

Satoshi Maeda[1], Minoru Morikawa[1], Shin-ichi Hasegawa[2], Jinchang Shao[2], Yui Kitayama[1], Masato Takao[3], Shinya Miki[3], Ken-ichiro Nakajima[3], Ken Innami[3], Koichiro Tsuji[2], Takashi Matsushita[3]
[1]Tokyo Lab/Office, Two Cells Co., Ltd., Tokyo, Japan, [2]R&D division, Two Cells Co., Ltd., Hiroshima, Japan, [3]Department of Orthopaedic Surgery, Teikyo University School of Medicine, Tokyo, Japan

Mesenchymal stem cells (MSCs) derived from synovium are widely
investigated for cartilage repair because of higher capacity in self-renewal
and chondrogenesis, comparing with MSCs from bone marrow and adipose tissue. In medical application, animal models are known to be crucial for preclinical evaluation of safety and effectiveness. However, larger animal models like canine haven't fully been investigated on preclinical effects of MSCs yet. In this study, we performed the characterization on cell surface markers of canine synovial MSCs and showed their differentiation potentials, using chemically defined serum-free media STK1® and STK2®, which we developed previously, comparing with DMEM containing 10 % fetal bovine serum (DMEM-10% FBS).Synovial membranes were picked out from rear knee joints of male

beagle dogs (11 months old) under arthroscopic surgery. They were then enzymatically digested with 0.4% collagenase at 37 °C for 90 min. The nucleated cells isolated from the tissues were cultured in STK1® in primary culture, and recovered as adherent cells. Subsequently, they were cultured in STK2® in subsequent passage culture. For osteogenic and adipogenic differentiations, the MSCs were changed to respective differentiation medium after confluency. For chondrogenic differentiation, MSCs were pelleted by centrifuge and then cultured in chondrogenic medium.The MSCs cultured by STK series media were adherent, spindle-shaped and fibroblast-like cells, expressing the pluripotency markers such as Oct3/4, Nanog, and Sox2 for about one month-expansion until passage 5 (P5). The MSCs cultured in STK series media showed higher proliferative potential, comparing with those grown in DMEM-10% FBS. In flow cytometric analysis in P3, these cells expressed surface markers, including CD29 and CD44, but did not detect CD14, 34, 45, or HLA-DR. Furthermore, it is interesting that either CD73 or CD105, defined as human MSC positive markers, was not detected. Osteogenic and adipogenic differentiations were demonstrated by staining with Alizarin Red S and Oil Red O in 21 day-cultures, respectively. Osteogenesis was also determined by elevated alkaline phosphatase activity. The cell pellets for chondrogesis were increased in size gradually and showed higher gene expression associated with the chondrogenic marker such as Sox9, Aggrecan, and Type II collagen in quantitative RT-PCR. These results indicate that the phenotype of canine MSCs derived from synovium is not necessarily consistent with that of human MSCs, and that the cytochemical stainings revealed that canine MSCs also have the multipotency in the same manner as human. In addition, it is found that their differentiation potential is improved by using STK1® and STK2®, comparing with DMEM-10% FBS. Because STK1® and STK2® are chemically defined serum free media, MSCs grown in these media are useful in in vivo studies on canine cartilage defect model, in order to obtain more obvious effectiveness with safety in transplantation.

W-3088

TAUROURSODEOXYCHOLIC ACID ATTENUATES LIPID ACCUMULATION AND ADIPOGENESIS OF MESENCHYMAL STEM CELLS BY MODULATING ER STRESS

Byung Hyun Cha, Jong Chan Ahn, Jin-Su Kim, Hee-Chun Kim, Sang Gyu Park, Soo-Hong Lee
Department of Biomedical Science, CHA University, Seongnam-si, Korea, Republic of

The obesity has become a serious health problem of modern people. Increased adipose mass in obesity is due to an increase in size (hypertrophy) of adipocyte and number (hyperplasia) of adipocyte. Chemical chaperone, tauroursodeoxycholic acid (TUDCA) not only has the ability to decrease endoplasmic recticulum (ER) stress, also plays a role as leptin-sensitizing agents at preadipocyte in the obese mice and human models. In this study, we examined whether TUDCA affects human mesenchymal stem cells (MSCs) such as bone marrow and adipose derived stem cells. To aim this, ER stress, lipid accumulation and adipogenesis of MSCs were observed by histological staining, RT-PCR and western blotting. Osteogenesis and chondrogenesis were also evaluated. TUDCA treatment significantly decreased ER stress and adipogenic differentiation of MSCs, however, did not affect osteogenic and chondrogenic differentiation. Likewise to preadipocyte, these demonstrate TUDCA plays a critical role to adipogenesis of MSCs by modulating ER stress. Thus, this novel finding suggests a new mechanism on MSCs to chemical chaperons such as TUDCA and would be useful for pharmacological and therapeutic applications against obesity.

W-3091

SINGULAR CELL CERAMIC CONSTRUCT MEDIATED OSTEOCHONDRAL INTERFACE ENGINEERING

Francis B. Fernandez, Sachin J. Shenoy, Suresh B. Babu, H K Varma, Annie John
Transmission Electron Microscope Lab, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram, India

Articular Cartilage damage is difficult to address with current surgical & therapeutic strategies. The native organization of hyaline cartilage tissue that renders it ideal for weight bearing & joint articulation via an avascular, low cell count structure also hinders healing. Post-intervention via conventional techniques generate fibrous tissue which is not commensurate in quality & integration with native tissue structures. The current study aims at understand-

ing adipose-derived stromal cell differentiation mediated by hydroxyapatite-based scaffolds that mimic bone structures to optimize & address cartilage defects caused by disease, trauma or degeneration.
Hydroxyapatite (HA) forms the native mineral component of bone & altered states of the same via a patented indigenous process is used as scaffold material -
(1) HASi - Hydroxyapatite coated with Silica
(2) BCP - Biphasic Calcium Phosphate (BCP) containing a graded ratio of HA to beta-tricalcium phosphate content.
HASi and BCP scaffolds were evaluated for its physico-chemical characteristics via Scanning Electron Microscopy (SEM), Fourier Transform Infra - red Spectroscopy (FT-IR) and X- Ray Diffraction (XRD). SEM images depicted the structural integrity and porous interconnected architecture (50 - 500 microns pore sizes) ideal for cellular infiltration and habitat. FT-IR detected the functional phosphate and silca groups and X-ray diffraction (XRD) pattern exhibited crystallinity with hydroxyapatite as the major phase in all the materials.
Adipose-derived stromal cells isolated from New Zealand White (NZW) Rabbit (RADMSC) were characterized, differentiated into the desired osteogenic & chondrogenic lineages and tested for compatibility with the prepared scaffolds. HASi and BCP proved to be non-cytotoxic and cytocompatible in direct contact with RADMSCs and their osteogenic & chondrogenic potential was utilized for defect healing.
For *in vivo*, cylindrical HASi and BCP tailored to defect dimensions were seeded with spatially segregated osteogenic & chondrogenic cells. Cell loaded scaffolds were implanted into 3.5mm (dia.) and 4mm (deep) defects in the medial femoral condyles of 6 month old NZW rabbits for 3 & 6 months. Defects at 6 months showed a lack of surface fibrillation or disarray as observed in similar long term weight bearing defect trials. Retrieved samples (3 months & 6 months post implantation) indicated progressive surface reorganization via Micro - CT (Contrast Enhanced - Hexabrix). Further details were elicited of near complete cartilage re-organization via stained plastic sections with material *in situ*. Sections were stained with Stevenal's Blue & Van Gieson's Picrofuchsin to demonstrate cartilage regeneration & bone formation. This was supported by studying integration of scaffolds into subchondral trabecular bone via Back Scatter Electron imaging
Studies on osteochondral reconstruction have popularly used bone marrow-derived mesenchymal stem cells in conjunction with polymeric scaffolds. Here, an attempt to combine easily accessible adipose-derived stromal cells in combination with a bone-integrating ceramic scaffold for smooth overgrowth of articular tissue is undertaken. The promising results open a unique pathway for future studies in this niche of novel bone supported cartilage reconstruction in Interfacial Osteochondral Engineering.

W-3092

CONTRIBUTION OF HUMAN MESENCHYMAL STROMAL CELLS TO DECELLULARIZED LUNG SCAFFOLDS

Julio J. Mendez, Mahboobe Ghaedi, Laura E. Niklason
Anesthesiology, Yale University, New Haven, CT, United States

Identifying appropriate cell sources to use in the recellularization of decellularized lungs is paramount in constructing a viable tissue engineered organ. To this end, we have investigated the potential of using human mesenchymal stromal cells as donor cells for tissue engineered lungs. Human bone marrow-derived mesenchymal stromal cells (hBM-MSCs) and human adipose MSCs (hA-MSCs) were isolated from freshly harvested bone marrow samples, or from abdominal fat lipoaspirate, respectively. FACS characterization of early passage cells demonstrated that both the hBM-MSCs as well as the hA-MSCs express the canonical MSC markers including CD90, CD105, and CD73, while being negative for CD45 and CD11b. Interestingly, both adipose and bone marrow derived MSCs also expressed markers associated with lung epithelium including CCSP, Pro-SPC and cytokeratin 5. In general, hA-MSCs contained fewer cells that were positive for both CCSP and pro-SPC following culture on plastic when compared with hBM-MSCs, whereas both had similar levels of cytokeratin 5 positive cells. MSCs from adipose and bone marrow were seeded onto decellularized rat lungs and cultured in a lung bioreactor for 7 days in small airway growth medium. After culture, both MSC sources resulted in robust coverage of the recellularized organ. Lungs recellularized with hBM-MSCs maintained expression of the type 2 pneumocyte marker, Pro-SPC as well as cytokeratin-5, while being negative for other lung epithelial markers including CCSP, p63, and caveolin 1. Co-culture experiments within the lung bioreactor allowed for the seeded cells to further differentiate towards cells that express markers of type 1

pneumocytes, including caveolin 1 and aquaporin 5, and more rarely to cells that expressed the Clara cell marker, CCSP. Together, these data indicate that mesenchymal stromal cells have the capacity for differentiation into lung epithelium when placed onto decellularized rat lungs, and further allows for the possibility of using MSCs as a potential cell source for tissue engineered lungs.

W-3093

PROTEOMIC PROFILING OF DIFFERENTIALLY EXPRESSED PROTEINS IN TOLL-LIKE RECEPTORS 3- AND 4-PRIMED MESENCHYMAL STEM CELLS

Mi Ran Choi, Mi Sook Doh, Sun Hwa Kim, Kyoung Hwa Jung, Young Gyu Chai
Department of Molecular & Life Sciences, Hanyang University, Ansan, Korea, Republic of

A variety of stimuli including inflammation stimulate expression of toll-like receptors (TLRs) in human bone marrow-derived mesenchymal stem cells (MSCs), resulting in the activation of TLRs' immune modulatory mechanisms. In the present study, MSCs were led to TLRs 3- and 4-primed cells by exposing them to poly(I:C) and LPS, respectively. And, the proteins up- and down-regulated in the cells were analyzed using proteomic techniques. We observed that rantes, IP10 and indoleamine 2, 3-dioxygenase were highly expressed in TLR3-primed cells, while IL6, IL8 and IL4 were highly expressed in TLR4-primed cells. Our proteomic analysis identified that 21 proteins including lysine degradation pathway associated proteins (2-oxoglutarate dehydrogenase precursor, procollagen-lysine,2-oxoglutarate 5-dioxygenase 2 isoform 1), focal adhesion proteins (type VI collagen alpha 2 chain precursor, filamen B) and peroxiredoxin 6 were up-regulated in TLR3-primed cells but down-regulated in TLR4-primed cells compared to MSCs. On the other hand, TLR3-primed cells showed the higher expression of proteolipid protein 2, pre-mRNA-processing factor 19, E3 ubiquitin-protein ligase LRSAM1 isoform 1 and liprin alpha4 proteins compared to TLR4-primed cells and MSCs. TLR4-primed cells showed down-regulated levels of neurabin-2, elongation factor 2 and heat shock protein 60 whereas vimentin and diacylglycerol kinase (theta) were up-regulated. These results show that stimulation of TLR3-priming in MSCs promotes the expression change of proteins responsible for protein polyubiquitination, cell motion and focal adhesion, while TLR4-priming induces the expression change of proteins associated with oxidation reduction and protein folding.

W-3094

ELONGATED STEM CELL MORPHOLOGY AND MATRIX STIFFNESS INFLUENCES LINEAGE BY MODULATING CONTRACTILITY

Ludovic G. Vincent
Bioengineering, UCSD, LA JOLLA, CA, United States

Myosin-mediated contractility has become a well-documented mechanism by which adult mesenchymal stem cells (MSCs) detect extracellular matrix (ECM) properties, e.g. stiffness, porosity (to control cell shape), etc., and respond by differentiating. ECM cues including stiffness and shape are usually synergistic, but fibrotic diseases alter stiffness, creating an imbalance in cues and thus aberrant contractility. We hypothesized that these cues could be "rebalanced" to a new optimum where appropriate myosin-mediated contractility is regained and MSC differentiation towards one lineage can be restored, i.e. highy elongated cells despite an abnormally stiff microenvironment to initiate myogenesis. To "rebalance" ECM cues, MSCs were plated on fibronectin patterns of varying aspect ratio but common area on compliant as well as abnormally stiff substrates that normally would or would not be permissive for myogenesis, respectively. MSCs expressed muscle specific myosin heavy chain (MHC) most strongly on compliant, muscle-like stiffness and elongated patterns with 10:1 and 15:1 aspect ratio. In contrast on abnormally stiff substrates, highest MHC expression corresponded to square and circle shaped cells. Cell contractility scaled with stiffness and decreased with increasing cell aspect ratio; square-shaped cells on abnormally stiff substrates, which were muscle MHC positive, produced the highest contractile energy rather than an optimal contractility observed for unpatterned MSCs on compliant substrates where muscle induction normally occurs. These data suggest that despite expression of muscle markers, contraction "rebalancing" is more complex than proposed. Indeed, expres-

sion and assembly of focal adhesion proteins such as vinculin, which transmit contractions, scaled more with cell shape than stiffness; highly elongated cells expressed the most vinculin but were the least contractile, suggesting that perhaps the force per focal adhesion could dictate differentiation via a focal adhesion-based sensor such as vinculin. However when induction media is used, differentiation was observed on all shapes and stiffness though highest when ECM and induction media were working synergistically towards one lineage. These data suggest that focal adhesion signaling pathways are unique to ECM-mediated induction, but overall, they suggest that lineage induction may be possible in non-permissive ECM niche, which could prove beneficial to treat fibrotic diseases.

W-3095

TRANSCRIPTION FACTOR NETWORK DYNAMICS IN EARLY OSTEOGENIC AND ADIPOGENIC COMMITMENT OF HUMAN MESENCHYMAL STROMAL CELLS

Jeroen van de Peppel[1], Tanja Strini[1], Marco Eijken[3], Peter van der Spek[2], Hans van Leeuwen[1]
[A]Internal Medicine, [B]Bioinformatics, [1]Erasmus MC, Rotterdam, Netherlands, [2]Arcarios bv, Rotterdam, Netherlands

Aging of the human skeleton is characterized by a decrease in bone mass due to increased bone resorption by osteoclasts and uncoupled bone formation by osteoblasts. A striking phenomenon is the increase of bone marrow adipocytes upon aging and in patients with osteoporosis. As osteoblasts and adipocytes share a common progenitor in the bone marrow (Mesenchymal Stromal Cells, MSCs), we wanted to understand the early regulatory events that occur upon differentiation of human MSCs. Identification of early transcription factors (TFs) and the regulation of target genes could expand our knowledge of lineage decision and commitment of hMSCs. Moreover, efficient differentiation will further improve the use of hMSCs in regenerative therapies.
To address this question, we have differentiated hMSCs into Osteoblasts (Dexamethasone , b-Glycerophosphate) and Adipocytes (Dexamethasone, IBMX, Indomethacin), isolated RNA and analyzed the transcriptome in a high temporal resolution using Illumina microarrays. Our analyses illustrate that gene expression changes upon differentiation of hMSCs into adipocytes or osteoblast were very dynamic. Based on these changes and unsupervised cluster analyses we could identify 3 differentiation phases within the first 4 days: 1) 0 to 3hrs, 2) 6hrs to 1 day and 3) 2 to 4 days. Interestingly, genes changing within the first phase were highly enriched for genes involved in transcription regulation, transcription factor activity and DNA-binding and suggested an important role for TFs in the initial differentiation phase.
To further investigate the TF network dynamics during lineage commitment, we concentrated on the TFs that changed their activity immediately upon differentiation (first phase). Using IPA's Upstream Regulator Analyses (Ingenuity Systems) we identified 33 TFs that changed activity within the first 3 hours of differentiation based on the regulated genes in this phase. Nine TFs were (in)-activated upon differentiation in both lineages and contained the dexamethasone activated glucocorticoid receptor and the cell cycle regulator CDKN2A. Four and 20 TFs changed activity when hMSC were differentiated into osteoblasts or adipocytes, respectively. Several of these TFs have not been described for their effects on osteogenic or adipogenic differentiation. However, knockout models described in literature have observed growth, skeletal, immune system and metabolic phenotypes that might be related to defective differentiation and lineage commitment of MSCs in vivo. Moreover, these TFs are associated with signaling pathways such as Glucocorticoid receptor, Aryl hydrocarbon receptor, Prolactin, ERK/MAPK and Wnt/b-Catenin signaling. Currently we are functionally characterizing the identified TFs in the initial phase of MSC commitment and will present here the effects of overexpression and interacting proteins of the identified TFs complexes.
Taken together, these in depth transcriptional analyses of differentiating hMSC increased our understanding of lineage decision/commitment. We have identified transcription factors that were (in)-activated upon differentiation of hMSC and can be key regulators for lineage decision holding potential for controlling bone formation.

W-3096

TRANSDIFFERENTIATION OF MOUSE ADIPOSE-DERIVED STROMAL CELLS INTO PRESUMPTIVE SALIVARY ACINAR CELLS BY CO-CULTURE SYSTEM

Jin-gu Lee, SangKyu Park, Sangho Roh
Cellular Reprogramming and Embryo Biotechnology Laboratory, College of Dentistry, Seoul National Univ., Seoul, Korea, Republic of

A irreversible loss of salivary gland function often occurs after radiation therapy in head and neck tumors, and transplantation of salivary acinar cells (SACs), in part, may overcome the side effect of the therapy. Recently, SACs were successfully differentiated from human amniotic epithelial cells (hAECs) and bone marrow stromal cells (BMSCs) by co-culture system and the cells are expressed the markers of SACs such as alpha-amylases, mucins and aquaporin 5. Here we used mouse adipose-derived stromal cells (ADSCs) as the cell source to differentiate directly into SACs using co-culture system. ADSCs have the ability of multipotency and can be easily collected from stromal vascular fractions of adipose tissues. The isolated ADSCs were cultured in DMEM supplemented with 20% fetal bovine serum (FBS, v/v), 1% non-essential amino acids and fibroblast growth factor-2 (5 ng/ml). The cultured ADSCs showed positive expression of their markers such as integin beta-1 (CD29), cell surface glycoprotein (CD44), endoglin (CD105) and nanog. The cells were able to differentiate into adipocytes after 2~3 week of culture and this was confirmed by lipid vesicles in their cytoplasm after Oil Red-O staining. Then, the ADSCs (2 X 104 cells/well) at passage 2 were co-cultured with mouse SACs (8 X 104 cells/well) in acinar's culture medium (DMEM/F12 with 20% FBS and 10 ng/ml epidermal growth factor) using the double-chamber system to avoid the mixture of those two types of cells, and the ADSCs were successfully differentiated to presumptive SACs in this co-culture system. Detailed results will be presented at the meeting. This study was supported by a grant from the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (MEST; 2012-0006145) and Technology Development Program for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry and Fisheries (MIFAFF; 111160-04), Republic of Korea.

W-3097

TRANSIENT VASCULARIZATION DIRECTS INTRINSIC CARTILAGE FORMATION FROM HUMAN PROGENITORS

Takanori Takebe, Shinji Kobayashi, Mitsuru Mizuno, Hiromu Suzuki, Yu-Min Chang, Emi Yoshizawa, Jiro Maegawa, Hideki Taniguchi
Yokohama City University, Yokohama, Japan

Craniofacial deformations caused by anomalies or traumas affect millions of patients worldwide. Current surgical interventions provide limited therapeutic outcomes; therefore, one promising approach involves the use of reconstructed cartilage grafts that utilize autologous mesenchymal progenitors. Adult cartilage is a unique avascular, aneural and alymphatic tissue. Despite the simplicity of this tissue, decades of study failed to direct homogenous cartilage tissues from transplanted mesenchymal progenitors. More specifically, only a limited population of progenitors successfully underwent mature chondrocyte differentiation in vivo, resulting in calcification or fibrocartilage formation. Here, we report that transient vascularization provides an important directional cue for progenitors to stimulate efficient chondrogenesis. Using an intravital imaging approach, we unexpectedly found that avascular cartilage development was promoted by initial vascularization and subsequent vessel regression. By recapitulating transient vascularization, we established a simple and highly efficient technique for regenerating human elastic cartilage. Elastic cartilage was quickly and efficiently grown from recently identified adult ear-derived human cartilage progenitor cells relative to conventional pellet culture transplants. By this approach, we generated three-dimensional (3-D) human elastic cartilage from progenitors without the use of the expensive exogenous factors or scaffolding materials that have been considered essential to the classical concept of tissue engineering. Cartilage regenerative therapies offer a promising approach for millions of patients with craniofacial deformations caused by anomalies or traumas, and the transplantation of vascularized cartilage progenitor cells provides a new potential method for efficient cartilage reconstruction in future clinical treatments.

W-3098

WNT ANTAGONIST sFRP4 INHIBITS THE ANGIOGENIC POTENTIAL OF HUMAN AMNION DERIVED MESENCHYMAL STEM CELLS

Sudha Warrier
Cardiovascular Regeneration, Manipal Insititute of Regenerative Medicine, Bangalore, India

The canonical Wnt/beta-catenin signaling pathway has been demonstrated to play an essential role in stem cell fate and in developmental angiogenesis. Genetic studies have implicated the Wnt/Frizzled (Fz) molecular pathway, namely Wnt7B and Fz4, in blood growth regulation. Mesenchymal stem cell (MSC) transplantation offers a robust source of angiogenic regeneration in vascular regenerative medicine. We have previously identified that MSCs from an avascular amnion have an inherent propensity for promoting angiogenesis by undergoing rapid vascularization and could be an ideal choice in wound healing and diseases that require rapid vascularization and tissue restoration. In the present study, we investigated the role of secreted frizzled related protein 4 (sFRP4), a modulator of the Wnt/Fz pathway, in regulating the angiogenic potential of amnion-derived MSCs (AMMSCs).
In our previous study, sFRP4 was shown to be a potent inhibitor of angiogenesis of endothelial cells. However, the effect of sFRP4 on MSCs with respect to their differentiation into the endothelial lineage has not been studied so far. Using various in vitro assays such as the endothelial ring formation assay, chemotactic assay and endothelial wound scratch assay, we established that sFRP4 inhibits endothelial cell differentiation and migration of AMMSCs. It also disrupted the stability of endothelial rings and retarded proliferation of MSCs. Mechanistic analysis of the action of sFRP4 was done by functional assays such as the effect of LiCl, quantification of ß catenin levels by Western blot, determination of intracellular calcium, superoxide levels, and catalase assays on sFRP4 mediated angiogenic inhibition of MSCs. From these analyses, we demonstrate that sFRP4 is able to block Wnt signaling via the canonical ß- catenin pathway. Furthermore, these functional data also reveal that sFRP4 also activates the Wnt/Calcium pathway. Induction of apoptosis of differentiated endothelial cells could be related to the activation of cellular reactive oxygen species.
These studies pave the way for understanding the role of sFRP4 in developmental angiogenesis. These results also suggest the use of sFRP4 as a potent angiogenic inhibitor of MSC mediated angiogenic differentiation in the context of the tumor microenvironment where pro-angiogenic factors secreted by MSC promote angiogenesis and tumor growth. Thus, targeting this interaction may lead to novel therapeutic and preventive strategies.

W-3101

GENERATION AND CHARACTERIZATION OF OOCYTE-LIKE CELLS DERIVED FROM HUMAN AMNIOTIC FLUID STEM CELLS

Xiaoli Yu, Huayan Wang
Dept. Biotechnology, Northwest A&F University, Yangling, China

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) cultured in appropriate environment have the ability to differentiate into oocyte-like cells (OLCs), which recruit adjacent cells to form follicle-like structures. OLCs were use to generate the live offspring after reconstituted with female gonadal somatic cells. However, the long term of transformation and the low efficiency to obtain OLCs have restricted their availability for experimental use. To improve the efficiency of OLC generation, human amniotic fluid stem cells (hAFSCs) were used as an oogenesis model. The $CD117^{+}/CD44^{+}$ hAFSCs showed the fibroblastoid morphology and has been propagated for more than 45 passages and maintained the multipotency and potential to differentiate into three germ layers *in vitro*. In addition to express stem cell markers of *OCT4*, *NANOG* and *SOX2*, hAFSCs also express germ cell markers such as *DAZL*, *SCP3* and *STELLA*. To generate OLCs, hAFSCs in early stage of passages were cultured in conditional media supplemented with 5% FBS and 5% porcine follicle fluid for 10-15 days. Cell aggregates were derived from hAFSCs, some of aggregates showed the structures that were morphologically similar to the primordial follicle, in which the estradiol production and the expression of steroidogenic enzymes had been proved. The cumulus-oocyte-complexes (COCs) structures were then ovulated from the aggregates, and the OLCs released from COCs were also observed. OLCs were in different sizes (range from 50-120 μm) with the thin and fragile zona pellucida, some of OLCs attached the surface of culture plate and some floating in the media. The folliculogenesis and oogenesis associated markers, including OCT4, VASA, FIGLA, IFTM3, DAZL, BMP15, GDF9, IFTM3, SCP3 and zona pellucida glycoproteins, were activated in both follicle-like structures and OLCs. Culturing OLCs in oocyte growth medium for another 5-10

days, some OLCs developed spontaneously into multi-cell structures similar to preimplantation embryos, which showed high cytoplasmic-to-nuclear ratio. Although the embryos were fragile, the embryo could be manipulated by the micropipette, suggesting that OLCs could be parthenogenetically activated at current culture condition, while the blastocyst-like structures were not discovered yet. In conclusion, hAFSCs retain the germ cell markers and can be transformed into OLCs in a short term (less then 20 days) and high efficiency *in vitro*. This study may provide a new desirable model for study germ cell formation due to wide range of sources and non-tumorigenicity.

W-3102

COL2.3GFP MARKED HUMAN PLURIPOTENT STEM CELLS DEMONSTRATE OSTEOBLAST SPECIFIC REPORTER EXPRESSION IN A MOUSE CALVARIAL DEFECT MODEL

Xiaonan Xin, Xi Jiang, Liping Wang, Mary Louise Stover, Shuning Zhan, Jiangping Huang, Liisa Kuhn, I-Ping Chen, Ernst Reichenberger, David Rowe, Alexander Lichtler
Reconstructive Sciences, University of Connecticut Health Center, Farmington, CT, United States

The use of hESC and induced pluripotent stem cells (iPSC) for study and treatment of genetic bone diseases, such as craniometaphyseal dysplasia (CMD) and osteogenesis imperfecta (OI), or bone injuries requires protocols to differentiate hESC/iPSC into cells with osteogenic potential. We have established zinc finger nuclease (ZFN) technology to increase the efficiency of homologous recombination at AAVS1 site, and have delivered a construct containing Col2.3 promoter driven GFPemerald to human embryonic stem cells (H9Zn2.3GFP), and Col2.3RFP to iPSC, to mark the cells in the osteoblast lineage. The targeted cells had a normal karyotype, and we demonstrated the pluripotency of a clonal line by Tra1-60 immunostaining, pluripotent low density RT PCR array and embryoid body (EB) formation. In a teratoma formed using H9Zn2.3GFP cells, we identified GFP positive osteoblasts and osteocytes. We observed GFP positive cells associated with alizarin complexone (AC) labeled newly formed bone surfaces. The GFP positive cells on the bone surface were also alkaline phosphatase (AP) positive, and we also saw GFP positive cells that were encased in the bone matrix, with the appearance of osteocytes. Immunohistochemistry with human specific bone sialoprotein (BSP) antibody has indicated that the GFP positive cells are also associated with human BSP containing matrix. This bone showed a distinctive pattern of AC labeling and bone structure, which was different from mouse bone. Therefore, we believe that our Col2.3GFP marks late stage osteoblast lineage cells. We have also obtained osteoblast specific expression using iPS cells targeted with Col2.3RFP. With these cell tools, we have tested two in vitro differentiation protocols. Following a published protocol (Boyd, et. at.), we cultured Col2.3GFP or RFP labeled hESC or iPSC as a monolayer in EGM media (EGM2MV with supplements, Lonza). The differentiated cells were implanted in mouse calvarial defects, where they produced relatively abundant new bone of human cell origin, as demonstrated by the same criteria used for the teratoma bone. This bone was formed without a cartilage intermediate, similar to membranous bone. We have also established a modified protocol, which was initiated by forming embryonic bodies (EBs). Mesenchymal cells were allowed to migrate out and proliferate from the EBs in EGM media. Using the cells derived from this EB differentiation protocol in the mouse calvarial defect model, we initially observed human chondrocytes, forming an extensive cartilage matrix, which were followed by human bone matrix formation. This is similar to endochondral bone formation. We expect that our cells with the Col2.3GFP or RFP reporter will be useful to develop optimized osteogenic differentiation protocols, and to isolate osteoblasts from normal and diseased iPSC for analysis.

W-3103

GENE EXPRESSION-BASED ENRICHMENT OF HUMAN ADIPOSE-DERIVED STEM CELLS FOR ENHANCED OSTEOGENIC DIFFERENTIATION

Hetal V. Desai, Eric M. Darling
Molecular Pharmacology, Physiology, and Biotechnology, Brown University, Providence, RI, United States

Introduction:

Human adipose-derived stem/stromal cells (ASCs) are an attractive source for cell-based therapies because of their lack of immunogenicity and applicability to musculoskeletal regeneration. However, ASCs display a heterogeneous response to stimuli during induction, which results in lower rates of differentiation. More homogeneous subpopulations of ASCs, however, may be predisposed to particular lineages. Isolating these subpopulations based on gene expression may provide an optimized cell source for regenerating specific tissues, and would greatly maximize cell yields while minimizing time in vitro.
The objective of this study was to isolate ASCs that are highly responsive to osteogenic induction factors based on expression of alkaline phosphatase (ALPL) mRNA, and to differentiate these cells in an effort to optimize osteogenesis.
Methods:
Expression of ALPL mRNA was detected in living cells using a DNA-based molecular beacon (MB), which yields a fluorescent signal upon binding its target mRNA. ASCs from the subcutaneous adipose tissue of seven healthy donors were obtained from Zen-Bio, Inc. and were passaged 4 times prior to use. ASCs were seeded in monolayer at a density of 25,000 cells/cm2. Cells were given osteogenic induction medium (OIM) containing lineage-specific growth factors. Four days into induction, the cells were treated with a custom-designed ALPL-specific MB via electroporation and subsequently sorted using FACS into positive (ALPL+) and negative (ALPL-) signal groups. A subset of beacon-treated cells from the same initial population was left unsorted.
Unsorted, ALPL+, and ALPL- cells were seeded in 96-well plates at 8,000 cells/well and given OIM. Following seven days of induction, four of the sample wells were lysed and assessed for units of alkaline phosphatase (ALP) protein activity (U). After 21 days of induction, the remaining four wells were fixed and stained with Alizarin Red-S (ARS), a charged dye which binds calcified matrix characteristic of bone formation. After staining, the dye was eluted and the absorbance (A) of the dye was measured at 540nm. The activity and absorbance measurements were normalized on a per cell basis (U/cell, A/cell).
Results and Conclusions:
Enhanced osteogenic differentiation existed in ALPL+ cells compared to either unsorted and ALPL- cells, resulting in significant increases in ALPL protein activity and deposition of calcified matrix.
ALPL+ cells had 1.4 times more ALP activity than unsorted cells (700 U/cell vs. 500 U/cell, $p<0.05$) and 14 times more activity than ALPL- cells (50 U/cell, $p<0.01$). Even more remarkably, ALPL+ cells displayed a two-fold increase in calcified matrix deposition over unsorted ASCs (A/cell=0.133±0.022 vs. 0.040±0.007, $p<0.01$) and a four-fold increase over ALPL- cells (A/cell=0.026±0.009, $p<0.01$).
Results indicated that sorting ASCs based on expression of ALPL yields subpopulations that are primed to undergo osteogenesis. Interestingly, though ASC populations contained only 10-15% ALPL- cells prior to sorting, removal of these unresponsive cells yielded disproportionately significant increases in osteogenic metabolite production. Pilot studies have shown similar trends for ASCs undergoing adipogenic differentiation when sorted using target genes such as lipoprotein lipase. Our reported findings show the utility of using FACS with MBs to obtain highly responsive cells for bone formation and could potentially be applied to other lineages.

W-3104

SUPPRESSION OF POLYAMINE BIOSYNTHESIS PROMOTE OSTEOGENIC DIFFERENTIATION OF HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

Mon-Juan Lee, Yo-Hsian Tsai, Kuan-Lian Lin
Department of Bioscience Technology, Chang Jung Christian University, Tainan, Taiwan

Polyamines are naturally occurring organic polycations that are ubiquitous in all organisms, and are essential for cell proliferation and differentiation. Although polyamines are involved in various cellular processes, their roles in stem cell differentiation are relatively unexplored. Results from our previous studies suggest that exogenous polyamines, including putrescine, spermidine, and spermine, were capable of promoting osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (hBMSCs). To investigate the mechanism underlying the osteogenic potential of polyamines and the crosstalk between pathways of osteogenesis and polyamine metabolism, we treated hBMSCs with α-difluoromethylornithine (DFMO), the irreversible inhibitor of the polyamine biosynthetic

enzyme, ornithine decarboxylase (ODC), to determine whether suppression of intracellular polyamine level affects the differentiation fate of hBMSCs. Our results indicate that DFMO up-regulated alkaline phosphatase (ALP) activity, and enhanced the mRNA expression of osteogenic genes such as Runt-related transcription factor 2 (Runx2), ALP, osteocalcin and osteopontin. In addition, extracellular matrix mineralization, a marker for osteoblast maturation, was accelerated in the presence of DFMO. We then suppressed the gene expression of ODC in hBMSCs using small interfering RNAs (siRNAs) designed against ODC (siODC), and found that the mRNA level of osteogenic genes such as osteocalcin and osteopontin were increased. These results suggest that suppression of polyamine biosynthesis may be correlated with the induction of osteogenic differentiation, and the level of intracellular polyamines may be manipulated to promote osteogenic differentiation. Currently, the only drug approved by the U.S. Food and Drug Administration (FDA) to stimulate bone formation is parathyroid hormone (PTH), which possesses the risk of inducing osteosarcoma. Studies on DFMO, a chemopreventive agent for cancer that is being evaluated in clinical trials, as novel osteogenic inducer not only help to elucidate the role of polyamine metabolism in the lineage commitment of stem cells, but also promote the development of polyamine-derived new drugs that stimulate bone formation.

W-3106

MULTIPOTENT PERIVASCULAR MESENCHYMAL STEM CELLS IN THE HUMAN BRAIN

Ilknur Ozen[1], Nicolaj Christophersen Christophersen[2], Thomas Reinbothe[3], Edward Visse[4], Patrik Brundin[2], Gesine Paul[5]

[A]Department of Clinical Science,Translational Neurology, [B]Department of Experimental Medical Science, Neuronal Survival Unit, [C]Diabetes Centre, Scania University Hospital Malmö, [D]Department of Clinical Science, The Rausing laboratory, [E]Department of Clinical Science, Translational Neurology, [1]Lund University, Lund, Sweden

Key questions in adult stem cell biology revolve around origin and physiological role of adult stem cell populations. Mesenchymal stem cells have remained elusive with regard to their *in vivo* physiology. Recent observations suggest that almost all adult tissues contain mesenchymal-like progenitors in the perivascular niche. These cells can differentiate into mesodermal cell types and may even be endowed with tissue-specific differentiation capacities. We have isolated and characterized a previously unrecognized progenitor cell population in the adult human brain. This cell population exhibits characteristics of both mesenchymal stem cells and pericytes *in vitro* (CD73, CD90, CD13, CD106, CD49d, PDGFR-, RGS5, -SMA, NG2) but in its native state does not express hematopoietic (CD34, CD45), endothelial (CD31), microglial (CD14, CD11b), glial (GFAP, O4), and neuronal progenitor markers (CD133, SOX1, NGN2, PAX6 and Musashi). We demonstrate, at a clonal level, that the progenitors have true multilineage potential not only towards a mesodermal but also neuroectodermal phenotype and can differentiate into neurons. Thus, the vasculature in the adult human brain contains progenitor cells with multilineage capacity that may represent a reservoir that can be exploited in attempts to repair the damaged or diseased brain.

W-3107

PERIODONTAL LIGAMENT DERIVED STEM CELLS EXHIBIT THE CAPACITY FOR LONG TERM SURVIVAL AND GENERATION OF MULTIPLE TISSUE TYPES IN VIVO

Danijela Menicanin[1], Krzysztof M. Mrozik[2], Victor Marino[2], Mark P. Bartold[2], Stan Gronthos[3]

[A]Dentistry, [B]CACDRC, Dentistry, [C]School of Medical Sciences, [1]University of Adelaide, Adelaide, Australia

Characterisation of progenitor cells residing in the periodontal ligament has demonstrated that these cells exhibit similar features to that described for bone marrow stromal cells (BMSCs) yet display specific distinguishing properties. Due to the developmental potential demonstrated by periodontal ligament stem cells (PDLSC), they are currently considered a favourable candidate for stem cell based tissue engineering applications for treatment of periodontal disease.

In this study we assessed the developmental capacity of ovine derived PDLSCs using an *in vivo* ectopic bone formation assay. BrdU labelled PDLSCs were subcutaneously transplanted into the dorsal surface of eight-week-old immunocompromised (NOD/SCID) mice. Concurrent with histological assessment of recovered PDLSC explants, progenitor populations were isolated and re-established from primary transplants, characterized *in vitro* and re-implanted into the NOD/SCID mice.
Our findings demonstrate the capacity of ovine derived PDLSC to survive, proliferate and generate multiple tissue types *in vivo*, including mineralised structures, fibrous tissue and vasculature. Furthermore, their ability to generate secondary structures highlights their potential for self-renewal and long-term contribution to tissue regeneration.
This study, characterising the growth and developmental properties of PDLSCs, contributes to the current body of knowledge and highlights the potential clinical use of these cells in tissue regeneration.

Muscle Cells

W-3111

REGENERATIVE CAPACITY OF OLD MUSCLE STEM CELLS DECLINES WITHOUT SIGNIFICANT ACCUMULATION OF DNA DAMAGE

Wendy Cousin, Michelle L. Ho, Rajiv Desai, Andrea Tham, Robert Y. Chen, Christian Elabd, Irina M. Conboy
Bioengineering, University of California, Berkeley, Berkeley, CA, United States

The performance of adult stem cells is crucial for tissue homeostasis but their regenerative capacity declines with age, leading to failure of multiple organs. In skeletal muscle this failure is manifested by the loss of functional tissue, the accumulation of fibrosis, and a reduced satellite-cell-mediated myogenesis in response to injury. While recent studies have shown that changes in the composition of the satellite cell niche are at least in part responsible for the impaired function observed with aging, little is known about the effects of aging on the intrinsic properties of satellite cells. For instance, their ability to repair DNA damage and the effects of a potential accumulation of DNA double strand breaks (DSBs) on their regenerative performance remain unclear.
This work demonstrates that there is no significant accumulation of innate DNA DSBs in muscle stem cells with age as assayed after cell isolation and in tissue sections. Moreover upon muscle injury, old satellite cells – known to have an impaired regenerative response – did not show any difference in the number of DNA DSBs at multiple time points after injury, in the expression of key DNA DSB repair proteins and DNA damage response genes, and in their radiosensitivity. Additionally, there is no significant difference in the expression of globally assayed DNA damage response genes, suggesting that not only DSBs, but also other types of DNA damage do not significantly mark aged muscle stem cells.
Interestingly, muscle stem cells from DNA-DSB-deficient SCID mice were as capable as those of young wild type mice to regenerate muscle *in vivo* and to form myogenic colonies *in vitro* despite an accumulation of DNA damage and a pronounced radiosensitivity, thereby suggesting that the inability to repair DNA DSBs does not directly correlate with the ability to regenerate muscle after injury.
Overall, our findings suggest that a DNA DSB repair deficiency is unlikely to be a major contributor to the decline in muscle regeneration observed upon aging.

W-3112

REVERSING THE AGING OF MUSCLE STEM CELLS BY SUPPLEMENTING GROWTH DIFFERENTIATION FACTOR-11.

Manisha Sinha[1], Juhyun Oh[1], Christine Miller[1], Samuel Regalado[1], Francesco Loffredo[2], Richard T. Lee[2], Amy J. Wagers[1]
[1]Stem Cell & Regenerative Biology, Harvard University, Cambridge, MA, United States, [2]Brigham and Women's Hospital, Cambridge, MA, United States

Aging is associated with a degenerative loss of skeletal muscle mass, strength, function and regenerative capacity after injury. Age-associated muscle wasting, weakness and slow recovery from injury profoundly impact health and

quality of life in the elderly human population. Skeletal muscle growth and regeneration after injury depend on a rare population of muscle fiber-associated mononuclear cells called satellite cells, which reside beneath the basal lamina and adjacent to the sarcolemma of myofibers. The precise molecular mechanisms that underlie age-related declines in muscle regeneration remain unclear, though several lines of evidence indicate both intrinsic cellular properties and extrinsic signaling factors as being responsible.
Amongst intrinsic properties, our data suggest that deficient muscle regeneration in old age results from a progressive decline in the overall frequency and function of satellite cells - the muscle-forming stem cells. Our work also implicates compromised genomic integrity in these cells as a potential mechanism by which age-dependent muscle dysfunction arises. Similar to other tissue-resident stem cells, age dependent compromise in DNA integrity in muscle stem cells may arise due to their quiescent nature under normal physiological conditions and their exposure to age-associated increases in oxidative and inflammatory stress, generated as by-products of normal muscle metabolism and local injury.
Importantly however, our data indicate that such age-related defects in muscle stem cell genomic integrity and myogenic activity may be reversible, and controlled at least in part by age-regulated systemic factors. The effect of cell-extrinsic systemic factors on reversing age-associated functional decline in adult stem and progenitor cells has been demonstrated in studies of the skeletal muscle and central nervous system after heterochronic parabiosis, a surgical intervention in which one old and one young mouse are joined and develop a shared blood circulation. From these studies, several soluble molecules, including members of the TGFb and Wnt super-family pathways, have emerged as “aging” factors that impede muscle regeneration.
In this study, we analyze the pro-myogenic effects of a youthful circulation and show that supplementing a systemic factor, Growth Differentiation Factor (GDF-11), in the circulation of aged mice can reverse the age-related impairment of muscle stem cell genomic integrity, myogenic function and regenerative capacity. Our data uncover a crucial mechanism of systemic rejuvenation of tissue stem cell function and identify a promising therapeutic candidate to encourage the maintenance and regeneration of aged skeletal muscle.

W-3113

IMMORTALIZED PATIENT MYOGENIC STEM CELLS RECAPITULATE PATHOGENESIS IN HUMAN CONGENITAL MUSCULAR DYSTROPHY DUE TO LAMININ-ALPHA2-DEFICIENCY.

SOONSANG YOON[1], GUIDO STADLER[2], WOODRING E. WRIGHT[2], JEFFREY B. MILLER[1]
[1]Department of Neurology and Physiology & Biophysics, Boston University School of Medicine, Boston, MA, United States, [2]Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX, United States

BACKGROUND. Congenital muscular dystrophy Type 1A (MDC1A) is a severe, recessive disease of childhood onset that is caused by mutation of the LAMA2 gene encoding laminin-alpha2. Studies with both mouse models and primary cultures of myogenic cells obtained from human MDC1A patients suggest that aberrant activation of cell death is a significant contributor to pathogenesis in laminin-alpha2-deficiency. Use of primary cultures to study MDC1A is constrained, however, both by the small number of available myogenic cell strains and the limited replication capacity of the primary cells.
OBJECTIVE AND METHODS. To determine if immortalized MDC1A myogenic cells would recapitulate the pathological changes seen in primary MDC1A cultures, we generated immortalized, clonal lines of human MDC1A and normal myogenic stem cells via overexpression of both CDK4 and the telomerase catalytic component (human telomerase reverse transcriptase; hTERT) using methods described previously (Stadler et al. 2011. Skelet Muscle. 1:12). We then compared proliferation, differentiation, and caspase-3 activation (as a marker of pathology) in cultures of immortalized MDC1A and normal vs. primary MDC1A and normal myogenic cells.
RESULTS. When cultured at low density in high serum growth medium, the immortalized MDC1A and normal myogenic cells maintained proliferation for >150 population doublings, whereas primary cultures ceased proliferating at 50 - 60 population doublings. In addition, at all population doublings tested, the immortalized MDC1A and healthy lines retained the capacity to fuse to form multinucleate myotubes and express muscle-specific genes when switched to low serum medium. Furthermore, activation of caspase-3, as assessed by immunostaining and en-

zymatic activity, was several-fold higher in the myotubes formed from immortalized MDC1A myoblasts compared to myotubes formed from immortalized control myoblasts. Upon treatment with staurosporine, caspase-3 activity was increased to a higher level in immortalized MDC1A than in control myogenic cells and Ku70 acetylation was also increased. This pattern of aberrantly increased caspase-3 activation in myotubes formed from immortalized MDC1A myogenic cells, was similar to that found previously in myotubes formed from primary MDC1A myogenic cells.
CONCLUSION. Immortalized MDC1A myogenic cells provide a new resource for studies of pathogenetic mechanisms and possible therapeutic approaches in laminin-alpha2-deficiency.

W-3114

MODULATING CELL CYCLE INHIBITORS TO CONTROL PROGENITOR CELL FATE DECISIONS

Pratiksha I. Thakore, David G. Ousterout, Charles A. Gersbach
Department of Biomedical Engineering, Duke University, Durham, NC, United States

A key step in the transition from a progenitor to a differentiated cell type is exit from the cell cycle. In mammals, most progenitor cells irreversibly lose proliferative capacity upon terminal differentiation. Cell cycle inhibitors, including cyclin-dependent kinase inhibitor (CKI) p21, retinoblastoma protein, and INK4a CKIs, orchestrate cell cycle exit and play a role in cessation of proliferation. These cell cycle inhibitors can thus be targeted to control whether cells remain progenitors or terminally differentiate. We hypothesized that reduced expression of p21 in a myoblast model would help the cells maintain a progenitor phenotype with increased regenerative potential by inhibiting differentiation and increasing self-renewal capacity. The CKI p21 is an ideal target for silencing because of a reduced risk of oncogenic side effects, compared to knockdown of tumor suppressors Rb and INK4. Also, the enhanced regeneration observed in MRL/MpJ mice and other “superhealer” strains is characterized by modified cell cycle activity and can be recapitulated in a p21 knockout mouse model. Using a lentiviral vector expressing short hairpin RNA (shRNA) against p21, we achieved over 85% knockdown of p21 expression in primary mouse myoblasts by quantitative PCR and western blot. In differentiation conditions, myoblast fusion into myotubes was dramatically reduced in p21 shRNA-expressing (p21sh) cells relative to non-treated and non-specific shRNA-treated controls. However, myogenic differentiation was not completely inhibited: p21 shRNA-expressing myoblasts showed similar upregulation of myogenic markers myogenin, troponin T, and desmin as non-treated and non-specific shRNA-treated cells after 3 days in differentiation conditions. This suggests that silencing p21 does not disrupt commitment to the myoblast lineage. Additionally, in a mixed population of p21sh and non-treated myoblasts, the p21sh cells are enriched 9-fold over 20 days in low serum culture. Silencing p21 allows progenitors cells to remain proliferative even in the absence of growth signals. This was further corroborated in BrdU-labeling studies, which indicated that a significantly higher percentage of p21sh myoblasts remain active in the cell cycle by progressing to S phase in low serum culture conditions. We have demonstrated that p21 expression can be controlled to effectively maintain a progenitor cell phenotype by delaying differentiation and preserving growth capacity. Furthermore, we have shown that modifying cell cycle inhibitors can increase the regenerative potential of these progenitor cells. When myoblasts were delivered to the tibialis anterior muscles of mice concurrently with cardiotoxin-induced injury, cells expressing p21 shRNA showed greater engraftment efficiency and fusion with the host muscle, compared both to non-treated and non-specific shRNA treated controls. Cell cycle inhibitors regulate terminal differentiation in a variety of cell types, and these findings demonstrate that modulating cell cycle inhibitor expression can be used to guide progenitor cell fate decisions. Preserving the regenerative capacity of progenitor cells can increase the efficacy of cellular therapies, in which expansion of donor stem cell populations without compromising proliferative potential or the progenitor phenotype is necessary.

W-3115

HUMAN ADIPOSE-DERIVED MESENCHYMAL STROMAL STEM-CELLS INJECTIONS IN GOLDEN RETRIEVER MUSCULAR DYSTROPHY (GRMD) DOGS: A FOUR-YEAR FOLLOW-UP

Mayana Zatz, Natassia M. Vieira, Mariane Secco, Eder Zucconi, Marcos Valadares, Carlos R. Bueno Junior, Mariz Vainzof, Mayra Pelatti, Juliana Gomes, Amanda Assoni, Thais Andrade, Vivian Landini
University of Sao Paulo, São Paulo, Brazil

Duchenne muscular dystrophy (DMD), a lethal X-linked disorder, is the most common and severe form of muscular dystrophies, affecting 1 in 3500 male births. Mutations in the DMD gene which result in the absence of muscle dystrophin are associated to a progressive and rapid degeneration of skeletal muscle. Affected patients are wheelchair-bound around age 10-12 and dependent for all activities before age 20. The possibility to treat DMD through cell therapy has been widely investigated. However before starting clinical trials in humans many questions still need to be addressed in pre-clinical studies. In addition to clarifying the role of the exogenous cells in the dystrophic muscle, one important concern is whether there is an increased risk of tumors or any other long-term side effects following stem-cells injections. Since mice models have a short life span, the best animal model to address questions related to the safety of the procedure is the Golden Retriever Muscular Dystrophy (GRMD) dog, which reproduces the full spectrum of human DMD. Affected animals carry a mutation that predicts a premature termination codon in exon 8 and a peptide that is 5% the size of normal dystrophin. These dogs present clinical signs within the first weeks of life and most of them do not survive beyond age two. We have injected human adipose-derived stromal cells (hASCs), from two different donors, in three GRMD dogs, without immunosuppression. Each animal was injected in the cephalic vein with 5X107 cells/kg within their first year of life: weekly in the first month and then monthly. Two of them (Dolar and Yuan) will be five years old in September and Rum is currently 1.6 years-old (born in july/2011). In Dolar and Yuan, who received cells from donor 1, we observed that hASCs injected systemically were able to reach, engraft, and express human dystrophin in the host GRMD dystrophic muscle up to 6 months after transplantation. When cells from donor 2 were injected in Rum, no human dystrophin was found in the host muscle but the three dogs showed clinical improvement during and right after the last hASCS injections with an apparent stabilization afterwards. Most importantly, after almost four years of follow-up for Dolar and Yuan there was no tumor or other side effect suggesting that hASCS transplantation is a safe procedure, which may have important applications for future therapy in patients with different forms of muscular dystrophies. Supported by FAPESP-CEPID,INCT-CNPq, ABDIM and the Duchenne research fund.

W-3116

EXPLORING THE MULTIPOTENT NATURE OF DEDIFFERENTIATED MUSCLE CELLS

Kinga Vojnits[1], Xiaodong Mu[2], Wei Xu[2], Yinda Tang[2], Haiying Pan[1], Yohan Choi[1], Yong Li[1]
[1]Pediatric Surgery, The Center for Stem Cell and Regenerative Medicine, The Brown Foundation Institute of Molecular Medicine (IMM) at the University of Texas Health Science Center at Houston, Houston, TX, United States, [2]The Laboratory of Molecular Pathology, Stem Cell Research Center, Childrens Hospital of UPMC, Pittsburgh, PA, United States

One of the striking phenomena in wildlife is the regrowth of damaged or wounded tissues. Unfortunately, mammals including humans have limited capacity for regeneration unlike several non-mammalian species, e.g. amphibians or zebrafish that can even replace complete limbs or renew their lost tissues. Similar evidences have also been discovered in the mammals, e.g. amputated digit tips of humans and mice can fully regenerate at early stages of development. The key feature in these processes is believed the dedifferentiation of mature cells into a more primitive, multipotent state. Here, we report the multipotent potential of injury-induced dedifferentiated mouse muscle cells, developed by our previously published cre/lox-β-galactoside system, which can be simply induced to neurosphere formation in vitro. Our results indicated these dedifferentiated cre/lox-β-galactoside positive cells formed easily and effectively neurospheres, floating in suspension, while the control primary myoblasts showed no sign forming these structures. Moreover, we present for the first time that the induced neurospheres expressed representative markers of all the three germ layers (such as Nestin, Ncam1, Brachyury, and Afp), indicating their heterogeneity as containing various cell types. After replacing these neurospheres into monolayer culture, their myogenic ability/memory recovered and formed myotube-like structures, highly expressing myogenic proteins (e.g. Myogenin and MyoD) and barely other lineage specific markers. Additionally, the neurospheres differentiated into the three major neural lineages (neurons, astrocytes, and oligodendrocytes) as they expressed Mtap2, β-Tubulin III, Gfap, Nestin and Olig1/2 on protein and gene level once cultured in a neural differentiation media. These findings suggest that dedifferentiated mammalian muscle cells are sensitive to environmental stimulation, having multipotent status, and can overcome germ lineage restrictions. Further work is needed to elucidate their survival, proliferation, migration and neuronal/myogenic differentiation capacity in vivo to prove their benefits in application of regenerative medicine for neural-muscle related injuries and diseases.

W-3117

EFFICIENT DERIVATION AND FACS PURIFICATION OF HYPAXIAL MUSCLE PRECURSORS FROM HUMAN PLURIPOTENT STEM CELLS

Bianca Borchin, Joseph Chen, **Tiziano Barberi**
Australian Regenerative Medicine Institute, Monash University, Clayton, Australia

Due to their intrinsic developmental potential, human pluripotent stem cells (hPSCs) provide an invaluable source of cells that could be used in regenerative medicine as well as an in vitro model to study early human development. Although significant advancements to the directed differentiation into specific neural (CNS, neural crest), endoderm (liver, pancreas) and lateral mesoderm (blood, endothelium) fates have been made, little has been attained on the derivation of skeletal muscle from hPSCs. Current protocols are inefficient or rely on transgene expression of specific myogenic factors to obtain the desired cells. Moreover, purification of early hPSC-derived muscle precursors has not been achieved.
With the aim of filling this gap, we set up a new method to specifically induce hPSCs to differentiate towards skeletal muscle in a monolayer feeder-free culture system. This highly efficient protocol has been based on a small molecule treatment of hPSCs for 4 days to promote skeletal muscle development, followed by a single growth factor treatment (10 days) for the expansion of the myogenic cells.
Starting at around day 20 of differentiation, in treated hPSCs, a population of Pax3+ muscle precursors started to be detected in the differentiating cultures and their number increased dramatically over the next 10 days. In contrast, this population was almost absent in differentiating untreated hPSCs. These Pax3+ cells after a few additional days started to co-express Pax7, another important muscle specifier gene, confirming their myogenic identity. In addition, we also detected on these cells the expression of Lbx-1, a homeobox transcription factors that marks migratory hypaxial muscle cells in the embryo. Importantly, we were able to purify by FACS the emerging Pax3+ as well Pax3+/Pax7+ precursors, using a combination of surface markers that included the chemokine receptor CXCR4. When replated, the sorted cells readily differentiated into myogenin+ myocytes or, under specific growth factor treatment, could have been expanded in culture and maintained undifferentiated for at least two additional weeks.
At around day 30-35 of differentiation, bipolar myocytes also started to be observed in the culture dishes of treated hPSC, with their number increasing over time. Thus, using another FACS strategy we were also able to purify directly these mature myocytes based on their expression of the Acetylcholin receptor (AchR). This additional strategy will enable investigators for a quick purification of hPSC-derived myocytes that could be immediately used for new drug and toxicity screenings.
Furthermore, we tested the ability of FACS purified muscle precursors to integrate in a host muscle upon transplantation of GFP-labeled cells in the limb buds of day 3.5 chick embryos. As expected, grafted cells started to migrate and differentiate along the host forming limbs.
This new differentiation protocol has been validated on several hPSC lines (ESC and iPS cells), including one derived from a muscular dystrophy patient.

The developmental progression from undifferentiated PSC toward the specification of muscle cells is largely unexplored in humans. The generation of myogenic precursors from normal and patient derived hPSCs, not only will give new insights into human skeletal muscle development and disease, but will also accelerate the move towards the use of hPSC-derived muscle cells for therapeutic use in a variety of muscle atrophy and wasting conditions.

W-3118

IN VITRO EXPANSION OF MOUSE SATELLITE CELLS FOR CELL THERAPY OF DUCHENNE MUSCULAR DYSTROPHY

Mohammadsharif Tabebordbar[1], Cong Xu[2], Leonard Zon[2], Amy Wagers[1]
[1]Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States, [2]Division of Hematology/ Oncology, Harvard Medical School, Children's hospital Boston, Boston, MA, United States

Duchenne Muscular Dystrophy (DMD) is a progressive muscle wasting disease caused by mutations in the *DYSTROPHIN* gene. Replacement of muscle stem cells (i.e., satellite cells) presents a promising therapeutic approach for restoring *DYSTROPHIN* expression to muscle fibers in these patients; however the low frequency of satellite cells in adult muscle is an obstacle for isolating sufficient number of cells for engraftment. To address this issue, we have sought culture conditions that would support the *ex-vivo* expansion of satellite cells and potentially provide more cells for transplantation. In this study, we screened 2,400 chemicals using a zebrafish blastomere culture system in order to find potentially conserved enhancers of muscle progenitor proliferation. Out of 6 chemicals that activated myogenesis in the zebrafish system, forskolin, an adenylyl cyclase activator, also dramatically expanded mouse satellite cells in culture. Forskolin treatment did not affect satellite cell survival, but did increase satellite cell proliferation by elevating cyclic adenosine monophosphate (cAMP) levels. Forskolin-treated cultured cells retained the immunophenotypic characteristics of engraftable satellite cells, and transplantation of compound-treated cells into dystrophic muscle yielded a significantly higher level of engraftment compared to control cells. Expansion of muscle satellite cells in culture using small molecule-based approaches such as that described here provides the possibility of improving cell replacement therapies for neuromuscular disorders.

W-3121

ENHANCED IN VIVO REGENERATIVE POTENTIAL OF FETAL MUSCLE STEM CELLS

Matthew Tierney, Francesca Boscolo, Alessandra Sacco
Sanford-Burnham Medical Research Institute, La Jolla, CA, United States

Adult muscle stem cells (MuSC) are required for the repair and homeostatic maintenance of skeletal muscle, residing in a quiescent state until stimulated by external cues to regenerate damaged tissues. Their developmental precursors, fetal MuSC, are an actively cycling population of Pax3/Pax7-expressing cells required for embryonic myogenesis and represent a related but intrinsically different cell population. To assess these cells functionally, we compared their self-renewal and regenerative potential with adult MuSC. Both populations were successfully isolated via flow cytometry using the cell surface markers alpha-7 integrin and CD34, uniformly expressed the paired box transcription factor Pax7 and were readily able to undergo terminal myogenic differentiation and form multinucleated myotubes in vitro. Interestingly, a subset of cycling fetal MuSC did not upregulate MyoD during in vitro expansion, suggesting that they are resistant to myogenic commitment. Cytokine exposure in vitro revealed that those uncommitted fetal MuSC may be preferentially sensitive to the potent mitogen FGF2, while also less responsive to Wnt/β-catenin signaling known to promote terminal differentiation. When transplanted in vivo intramuscularly, fetal MuSC displayed a robust proliferative potential and successfully fused with a significantly higher number of resident myofibers than adult MuSC. Following engraftment, fetal MuSC were able to give rise to an expanded pool of progenitors following serial injury and also retain their regenerative potential following serial transplantation, demonstrating long-term self-renewal capacity. Finally, we observed that the progressive entry of Pax7+ MuSC into the satellite cell niche is associated with reduced proliferation, suggesting that the changing microenvironment during developmental myogenesis plays a critical role in MuSC function. Our current work continues to utilize comparative studies aimed at uncovering the molecular pathways responsible for governing fetal MuSC regenerative

behavior. In addition, we are pursuing the use of genetic models to dynamically study clonal patterning and fate mapping of MuSC to improve our understanding of the factors governing MuSC behavioral changes during development.

W-3122

ENGINEERING A WNT7A-BASED PROTEIN THERAPEUTIC FOR THE TREATMENT OF MUSCULAR DYSTROPHY

Monica H. Bennett[1], Tom Lee[1], Kevin Lai[1], Michael Fitch[1], Christina Lynch[1], Michael Rudnicki[2], Peter Flynn[1]
[1]Fate Therapeutics, San Diego, CA, United States, [2]Department of Medicine, University of Ottawa, Ottawa, ON, Canada

In healthy skeletal muscle, satellite stem cells are the key regenerative precursors: normally quiescent but becoming activated, proliferating and differentiating into myoblasts for either de novo fiber formation or as the source of increased nuclear content for existing fibers in response to tissue damage. A core pathophysiologic phenomenon seen in muscular dystrophy is a cycle of muscle degeneration leading to continuous compensatory satellite cell activation and differentiation to affect a regenerative response. It is clear that the regeneration cannot keep pace, and tissue degeneration results in ultimate loss of function. Identifying and enhancing the underlying molecular and cellular mechanisms of the natural tissue repair process by restoring the balance of quiescent and activated stem cells thus represents a promising approach to therapeutic intervention, irrespective of etiology. WNT7a has previously been established as a potent mediator of muscle regeneration through a combination of satellite stem cell symmetrical expansion and direct myofiber hypertrophic activity. In recent data, wild-type WNT7a protein treatment has been shown to confer significant functional improvement in rodent models of muscular dystrophy. Wnt proteins are developmental morphogens that drive embryogenesis, stem cell fate determination and cellular proliferation and differentiation in a tissue/cell-specific manner. Wnt proteins have not previously been developed as therapeutics primarily due to challenges in protein manufacture and formulation at scale. Building on the academic observations of WNT7a activity, we have used rational protein design and structural engineering to produce a panel of WNT7a analogs with preferred pharmaceutical, manufacturing and formulation properties. These WNT7a analogs induced significant myotube hypertrophy in vitro using murine, human DMD, BMD and FSHD primary myoblast culture systems. The direct administration of the pharmaceutical candidate WNT7a proteins to the tibialis anterior (TA) muscle of either wild-type or dystrophic (MDX) mice resulted in significant satellite stem cell expansion and fiber hypertrophy. In histological assessments, treatment with the engineered WNT7a protein forms significantly reduced inflammation and muscle fiber necrosis in the MDX mouse. Further, in situ measurements demonstrated significant increases in specific force and twitch force on WNT7a-analog administration to the MDX TA muscle. Together, these results suggest the therapeutic potential for WNT7a and demonstrate a clear path to drug candidate development.

Cardiac Cells

W-3131

PHENOTYPIC REVERSAL OF LONG QT SYNDROME 2 IN PATIENT-SPECIFIC HUMAN PLURIPOTENT STEM CELL DERIVED CARDIOMYOYCTES

Ashish Mehta[1], Glen Lester Sequiera[1], Chrishan JA Ramachandra[1], Yuliansa Sudibyo[1], Yingying Chung[1], Sheng Jingwei[1], YW Wong[2], TH Tan[2], Reginald Liew[3], Philip Wong[1], Winston Shim[1]
[1]National Heart Centre Singapore, Singapore, Singapore, [2]KK Hospital, Singapore, Singapore, [3]DUKE-NUS Singapore, Singapore, Singapore

Long QT Syndrome 2 (LQTS2) caused by missense mutations in hERG channel is clinically associated with abnormally prolonged ventricular repolarization and sudden cardiac deaths. Modeling monogenic arrhythmogenic dis-

eases using induced pluripotent stem cells (iPSCs) offer unprecedented mechanistic insights into disease pathogenesis.
Aim: We evaluated if cardiomyocytes (CMs) derived from LQTS2 patient-specific (A561V mutant) hiPSC recapitulate the disease phenotype with defective trafficking of hERG channel in vitro and demonstrate phenotypic reversal through pharmacological intervention.
Methods: Electrophysiological recordings through microelectrode arrays (MEA) and patch clamp were utilized to recapitulate disease phenotype as well as I_{Kr} current recordings. Gene expression arrays and immunofluorescence were performed to demonstrate defective and corrective trafficking of hERG in LQTS2-hiPSC derived cardiomyocytes.
Results: We produced cardiomyocytes manifesting LQTS2 phenotype (A561V missense mutation in *KCNH2*) from iPSCs using virus-free reprogramming method. These cardiomyocytes recapitulate dysfunction of hERG potassium channel with 90% diminished I_{Kr} currents, 30% prolonged repolarization durations and elevated arrhythmogenesis. Further, our results demonstrated 3-fold reduction in hERG sarcolemma localization densities together with a 4-fold increase in sequestered hERG within the ER-golgi complex. This was also accompanied with significant dysregulation of folding chaperones and processing proteasome markers. These results collectively confirmed trafficking defect-induced disease manifestation. Treatment with a calpain inhibitor and atypical 26S proteasomal inhibitor significantly reduced disease manifestation by enhancing I_{Kr} currents, reducing repolarization durations and diminishing arrhythmogenesis. This pharmacological intervention also resulted in increasing sarcolemmal hERG localization densities with reduced sequestering within the ER-Golgi complex. Diverged from biophysical interference of hERG channel, our results show that modulation of chaperones and proteasomes could re-direct hERG channel to sarcolemmal membrane and reduce prolonged repolarization durations in LQTS2-CMs.
Conclusion: Understanding the molecular mechanisms governing arrhythymogenic diseases could be effectively utilized for early evaluations of candidate drugs that could be helpful in developing therapeutic regimes for LQTS2. Our study for the first time leverages the potential of reprogramming technology in disease modeling for an alternative mechanistic elucidation of rescue strategy for LQTS2 via corrective re-trafficking.

W-3132

PURIFICATION OF LONG QT-SYNDROME 3 INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES FOR PHARMACOLOGICAL SCREENING WITH AUTOMATED PLANAR PATCH CLAMP

Daniela Malan, Stephanie Friedrichs, Bernd K. Fleischmann, Philipp Sasse
University of Bonn, Bonn, Germany

Long QT syndrome 3 (LQTS 3) is an inherited disease due to a gain of function mutation of the cardiac sodium channel (Scn5a). This disease is characterized by a prolonged long QT interval in the ECG and patients suffer from life threatening cardiac arrhythmias and sudden cardiac death. To show that induced pluripotent stem (iPS)-cells can be used to investigate this disease in vitro, we have generated iPS cells from a LQTS 3 mouse model carrying the human ΔKPQ deletion of Scn5a. Cardiomyocytes differentiated from these LQTS3-iPS cells showed faster recovery from inactivation of sodium currents and larger late sodium currents compare to wild-type cells. Moreover action potential durations were prolonged and early afterdepolarizations occurred at low pacing frequencies in LQTS 3-specific iPS derived cardiomyocytes.
For screening of pharmacological substances using automated assays, a pure population of cardiomyocytes is required. We have therefore genetically engineered wild-type and LQTS 3 iPS cell lines with a lentivirus to expresses a puromycin-resistance gene under the control of the α-myosin heavy chain promoter. Application of puromycin to differentiated iPS cells led to death of non-cardiomyocytes and resulted in a nearly pure cardiomyocyte population (wild-type: 92.8±6.2 %, n=5, and LQTS 3: 87.7±9.7%, n=4). Purified LQTS 3-specific cardiomyocytes showed also prolonged action potential durations and early afterdepolarizations at low pacing rates. Furthermore the Na+ channel blocker mexitilene showed a reduction of action potential duration in purified LQTS 3-specific cardiomyocytes.
To prove the concept that these cells could be used for drug screenings, we performed automatic planar patch clamp analysis (Nanion Technology) with purified wild-type and LQTS 3-specific cardiomyocytes. Voltage ramps

showed the typical inward and outward currents of iPS-derived cardiomyocytes. Action potential were measured automatically at different pacing frequencies and LQTS-specific cardiomyocytes showed the characteristic prolongation at low rates.
Hence, LQTS 3-specific cardiomyocytes can be purified form disease-specific iPS with a lentiviral strategy, maintain the specific hallmarks of the disease and are suitable for automated drug screening by planar patch clamp analysis.

W-3133

ISOLATION OF HUMAN C-KIT+ CARDIAC PROGENITOR CELLS TO IMPROVE OUR UNDERSTANDING OF CARDIAC TRANSCRIPTIONAL REGULATION

Claire N. Medine, Judy Fletcher, Franziska Sendfeld, Nicholas L. Mills, Ian Wilmut
MRC Centre for Regenerative Medicine, The University of Edinburgh, Edinburgh, United Kingdom

It was previously believed that the heart was a terminally differentiated organ, with limited or no regenerative capacity. However, over the last decade compelling evidence suggests that the heart does undergo regeneration with a limited population of resident progenitor cells able to replenish cardiomyocytes lost due to injury throughout adult life. The therapeutic manipulation of resident cardiac progenitor cells could transform outcomes in patients with heart failure if these cells can be harnessed for myocardial regeneration. In recent years, the search for cardiac progenitor candidates has gathered momentum, and several populations of resident cardiac progenitor cells have now been isolated in rat, mouse, dog, porcine and human hearts using a variety of stem cell markers. With a variety of cardiac progenitor populations now described it is important that we fully understand the properties and differentiation potential of these progenitor populations. We describe the isolation of c-kit+ potential cardiac progenitor cells from atrial appendages of patients undergoing coronary artery bypass or valve replacement surgery (n=6) and from human fetal hearts (n=6). C-kit+ magnetic activated cell sorting (MACS) was used to isolate c-kit+ cells that were expanded in culture. These populations were CD45-, CD34-, but positive for CD146, CD105, KDR and the early cardiac transcription factors Mef2c, GATA4, Nkx2.5 and Isl1. The c-kit+ populations were able to differentiate in vitro to cardiomyocytes and endothelial cells. To improve our understanding of the dynamic networks of transcriptional regulation in cardiac progenitor cells both fetal and adult c-kit+ cardiac progenitors were used as a tool for gene profiling to identify key genetic differences between the populations. Single-cell gene expression techniques also allowed us to detect variations among cells in seemingly homogenous populations. This allowed us to detect overlapping and non-overlapping expression patterns and thus provide novel insights into the complex networks of transcriptional regulation in cardiac progenitor cells. Understanding the transcriptional regulation of cardiac progenitor cells will identify novel approaches for cellular reprogramming and permit us to develop robust methods for isolating and expanding these cells for clinical application in the treatment of patients with heart failure.

W-3136

Gene Signatures of Heart Chamber Myocytes Identify Chamber-Specific Cardiac Progenitors

Peter Andersen[1], Laviel Fernandez[2], Hideki Uosaki[2], Chulan Kwon[2]
[1]Johns Hopkins Univ, Baltimore, MD, [2]Johns Hopkins Univ, Baltimore, MD, United States

Pluripotent stem cell (PSC)-derived cardiomyocytes have emerged as a promising cell source to repair damaged myocardium. However, due to the lack of molecular and functional information of early cardiomyocytes, it remains unclear which types of myocytes are generated from PSCs. Here, we isolated atrial, right ventricular, and left ventricular myocytes from embryonic hearts and examined their properties by microarray and patch-clamp analyses. We found that a large number of genes are differentially regulated in each of the chamber myocytes, including genes encoding transcription factors, surface receptors and channel proteins. Moreover, they exhibited distinct electrophysiological properties. PSC-derived myocytes were composed of cells with properties of the embryonic atrial, right ventricular, and left ventricular myocytes, indicating myocardial heterogeneity at molecular and functional levels. Strikingly, some of their gene signatures remained in early cardiac progenitors and this allowed us to purify cardiac progenitors giving rise to each chamber cells. This study provides defined parameters to distinguish

cardiomyocytes of different chambers and to isolate chamber-specific cardiac progenitors from PSC systems, which will be instrumental for cell transplantation therapy and for diagnosis and drug screening of heart diseases.

W-3137

TREATMENT WITH THE HISTONE METHYLTRANSFERASE INHIBITOR BIX01294 ENHANCES THE CARDIOPOTENCY OF BONE MARROW STEM CELLS

Jinpu Yang[1], Carol A. Eisenberg[2], Leonard M. Eisenberg[2]
[1]New York Medical College, valhalla, NY, United States, [B]Physiology, [1]New York Medical College, valhalla, NY, United States

Heart disease is the leading cause of death in the US. Since the optimal treatment for the damaged or diseased heart is to replace dysfunctional myocytes, great effort has been made to develop stem cell therapies for regenerating cardiomyocytes. Bone marrow (BM) has long been considered a potential stem cell source for cardiac repair due to its abundance and accessibility; however, the capacity of this tissue to generate cardiomyocytes has been low. To improve the cardiac competency of BM cells, we screened various pharmacological reagents and molecules that were previously shown to assist the acquisition and/or maintenance of stem cell pluripotency, for their effect in broadening the differential potential of BM cells. Among the pharmacological reagents we screened, only BIX01294, a selective inhibitor of G9a histone methyltransferase (HMTase), was able to reprogram BM cells to a cardiopotent phenotype. This finding was indicated by upregulation of genes identified with precardiac progenitors (Brachyury, Mesp-1 and Isl-1). Targeting G9a HMTase by shRNA knockdown provided supporting evidence that the expression of these cardiac progenitor markers resulted from the inhibition of this enzyme. Time course studies established that 48 hr treatment with 8μM BIX01294 was optimal, and that the subsequent removal of the drug allowed cells to differentiate in response to the cardiogenic inducing factor Wnt11. When pretreated with BIX01294, BM cells exhibited a more profound response to Wnt11, as shown by the high level expression of myocardial genes and proteins, including Nkx2.5, Gata4, Hand1, Hand2, Tbx5, myocardin, β-MHC, α-actinin, and titin. In addition, we found that Trichostatin A (TSA), a broad inhibitor for histone deacetylases (HDACs), and the DNA demethylating agent 5-azacytidine were able to synergize with BIX01294 in promoting cardiac gene expression by BM cells. In summary, we report that inhibition of histone methyltransferase using BIX01294 reprograms BM cells and broadens their cardiac potential. These data indicate that treatments with pharmacological inhibitors that promote specific epigenetic modifications may have utility for developing stem cell sources for cardiac repair and regeneration.

W-3138

THE COMBINED TRANSPLANTATION OF TWO DIFFERENTLY-PROCESSED HUMAN SECONDARY CARDIOSPHERES IS THE OPTIMAL STRATEGY FOR CARDIOVASCULAR REGENERATION AND INFARCT REPAIR.

Hyun-Jai Cho, Ho-Jae Lee, Ju-Young Kim, Jooeun Lee, Sahmin Lee, Han-Mo Yang, Yoo-Wook Kwon, Hyo-Soo Kim, Young-Bae Park
Seoul National Univ, Seoul, Korea, Republic of

Background: Adult heart possesses resident cardiac stem or progenitor cells. Of them, *ex vivo*-expanded cardiosphere-derived cell (CDC) is one of promising candidates to repair damaged hearts. We previously reported that the generation of murine and human secondary cardiospheres from CDCs is the effective cell processing strategy to improve the therapeutic efficacy. Compared with monolayer-cultured CDCs, both Oct-4, (a cellular potency marker) and VEGF (a paracrine marker) was markedly up-regulated during secondary CS formation. However, enhancing the cardiopoietic regenerative potential of cultured cells would be irreconcilable with promoting the secretory activity of cells for paracrine humoral effects.
Objectives: We aimed to optimize cell processing strategy to escalate regenerative

potentials as well as to enhance paracrine effects. We hypothesized that the combined approach using two different processing and mixed transplantation thereafter would be desirable to maximize therapeutic benefits.
Methods and Results: During secondary CS formation, we found that ERK pathway played a directional role toward cellular potency or paracrine capacity. Therefore, we generated secondary CSs using two different methods; ERK activation and inhibition. When we stimulated the ERK pathway during secondary CS generation by adding EGF, TGFβ and bFGF in medium, VEGF expression was upregulated. Conversely, when we blocked the ERK pathway by adding receptor tyrosine kinase-1 (RTK-1) and TGFβ blockers and withdrawing EGF in culture medium, Oct-4 expression was increased up to 20-folds.
Next, to trace cells *in vivo*,
we tagged two differently-processed secondary CSs by green and red nanoparticles, respectively, and transplanted 3 x 10^5 cells (1.5 x 10^5,
each) into the infarcted heart of athymic nude mice. Three days after myocardial infarction and cell injection, we confirmed human VEGF secretion in the tissue using human specific VEGF antibody. VEGF was mainly secreted from the secondary CS processed under ERK stimulating condition. Interestingly, we observed secondary CS-derived cardiomyocyte-like cells in the peri-infarct zone at day 14. Cardiomyocyte-like cells were mainly developed from the secondary CS processed under ERK inhibition condition.
Compared with the injection of PBS, CDCs and secondary CSs generated by previous single culture condition, the combined transplantation of two differently-processed secondary CSs markedly increased capillary density at day 14. Furthermore, echocardiography showed that LV dimensions at both systole and diastole were significantly smaller while LV systolic function was greater in the combined transplantation group. In accordance with the physiologic data, Masson's trichrome staining revealed LV thickness and dimension were preserved and fibrotic scar area was reduced in the combined transplantation group.
Conclusions: We demonstrate that ERK pathway plays a directional role in the cell processing *in vitro* and the combined transplantation
of two differently-processed human secondary CSs is a promising strategy to maximize therapeutic benefits through enhancing cardiopoietic regenerative potentials and promoting paracrine humoral effects.

W-3141

LINEAGE-SPECIFIC MOSAICISM IDENTIFIES RENEWING CARDIAC PROGENITOR CELLS.

Lincoln T. Shenje, Peter Andersen, Hideki Uosaki, Laviel Fernandez, Peter Rainer, Dong Lee, David Kass, Chulan Kwon
Medicine, Johns Hopkins School of Medicine, Baltimore, MD, United States

Cardiac progenitor cells (CPCs) must make the proper decisions to control their number to sustain the rapid heart growth. However, it is unknown if stem cell-like CPCs are available for the process. Here, we demonstrate the presence of a small number of renewing CPCs (rCPCs) and their niche, where CPCs remain proliferative and change fates as they exit the niche. Genetic ablation of the cell-fate regulator Numb and its homologue Numblike depleted CPCs in this niche, leading to an atrophic heart and embryonic lethality. We rescued the lethality by lineage-specific mosaicism and traced CPCs lacking Numb and Numblike. Mutant CPCs normally differentiated into cardiovascular cells, but failed to become rCPCs. The phenotype was recapitulated by deleting β1-integrin, a conserved stem cell regulator that is associated with Numb. Our study indicates that rCPCs serve as a renewable source of CPCs and may provide a stem cell-niche paradigm for heart biology.

W-3142

A METHOD FOR THE ISOLATION OF PHENOTYPICALLY STABLE SELF-RENEWING CARDIAC STEM CELL LINES

Georg Weitzer
Max F. Perutz Laboratories, Medical University of Vienna, Vienna, Austria

Preparative FACS has generated numerous cell populations from heart tissues which have been characterized as somatic stem cells. However, none of these cell populations characterized and isolated by the presence of markers such as Sca1, c-Kit, Isl1, or CD34 could be subcloned and indefinitely maintained as a phenotypically stable stem cell line so far.
Here we present a method to obtain stable clonally derived stem cell lines from hearts of different age by coculture of triturated heart cells with embryonic stem cells and mitotically inactivated SNL76/7 fibroblasts secreting leukemia inhibitory factor. This mixture of cells creates an environment which mimics a stem cell niche allowing the survival and leukemia inhibitory factor-dependent proliferation of cardiac stem cells. These cardiovascular progenitor cells are enriched by a modified 3T3 protocol and after that embryonic stem cells are removed by either positive or negative selection. 17 cardiovascular progenitor cell lines were independently generated from whole hearts of 2, 22, and 180 day old C57BL/6Jx129Sv and Balb/c mice. The remained diploid over up to 149 passages and exclusively differentiated to cardiomyocytes, smooth muscle cells, and vascular endothelial cells.
While self-renewing they all express the stemness transcription factors Oct4, Sox2, Nanog, and Tert, and at the same time, significant levels of mesodermal and early myocardial transcription factors, Brachyury, Mesp1, Nkx2.5, Isl1, and Mef2C. Neither the presence of activin A nor the presence of low levels of retinoic acid induced differentiation to any endodermal or neuro-ectodermal cell types. Differentiation to atrial, ventricular, and pace-maker cardiomyocytes was enhanced in the presence of Bmp2 and leukemia inhibitory factor attenuated the Bmp2-dependent cardiomyogenesis in a dominant negative manner. Finally removal of SPARC (secreted protein acidic and rich in cysteine), which is secreted by the cardiovascular progenitor cells, significantly, inhibits cardiomyogenesis, and when added induces the temporal upregulation of Brachyury and Nkx2.5 in differentiating cardiovascular progenitor cells.
These results demonstrate the feasibility of the isolation and indefinite maintenance of murine cardiac stem cells, and suggest SPARC as a new player in the regulation of cardiomyogenesis.
Acknowledgment: These is a summary of the work and diploma theses of undergraduate students Julia Hoebaus, Philipp Heher, Teresa Gottschamel, Matthias Scheinast, Harmen Auner, Diana Walder, Marc Wiedner, Jasmin Taubenschmid, and Maximilian Miksch and has been funded by the Austrian Science Fund (FWF), grant P18659, the Herzfelder´sche Familienstiftung, and the Hochschuljubiläumsstiftung der Stadt Wien, grants H-2174/2007, H-1249/2009, and H-2302/2011 to G.W.
Parts of these data are published in
Hoebaus, J., Heher, P., Gottschamel, T., Scheinast, M., Auner, H., Walder, D., Wiedner, M., Taubenschmid, J., Miksch, M., Sauer, T., Schultheis, M., Kuzmenkin, A., Seiser, C., Hescheler, J., Weitzer, G. Embryonic Stem Cells Facilitate the Isolation of Persistent Clonal Cardiovascular Progenitor Cell Lines and Leukemia Inhibitor Factor Maintains their Self-Renewal and Myocardial Differentiation Potential In Vitro. (2013). Cells Tissues and Organs. Published online on 01/22/2013; DOI: 101159/000345804

W-3143

ENGINEERED STRATIFIED CELL SHEET BASED ON ENDOGENOUS CARDIAC STEM CELLS AND MYOCARDIUM-LIKE EXTRACELLULAR MATRIX FOR MYOCARDIAL REGENERATION

Heechul Kim[1], Chung Mu Park[2], Jong Tae Kim[1], Hyeong-In Kim[1], Young-Il Yang[2]
[1]Paik Institute for Clinical Research, Inje University, Busan, Korea, Republic of, [2]Inje University School of Medicine, Busan, Korea, Republic of

Endogenous cardiac stem cells (CSCs) provide a promising avenue to regenerate the injured myocardium due to their intrinsic propensity to differentiate into all cardiac cells. However, critical issues including transplantation unit and route should be overcame for translating into clinical application of these cells. Cell sheet engineering using temperature-responsive polymer and cardiomyogenic precursor cells has been approached for myocardial regeneration. This approach appears useful, but cell sheet reveals limitations, such as lack of cell-to-extracellular matrix (ECM) interaction, mechanical fragility, and requiring a complex manipulator to stack single-layered cell sheet into multi-layered cell sheet. To overcome these limitations, we developed a novel one-step method to fabricate a stratified cell sheet based on 3-dimensional (3D) cell culture in the fibrin hydrogel and cell-mediated fibrinolysis and compaction. The fibrin hydrogel provides a temporal matrix for 3D spatial distribution of CSCs. Under stressed culture condition, CSCs begun to rapidly spread, bind to each other and form cellular network throughout the 3D hydrogel. The extracellular space between CSCs and the fibrin revealed the deposition of newly synthesized ECM that composed by collagen type I and IV, laminin, and fibronectin and replaced the preexisting the fibrin hydrogel. Moreover, proangiogenic mRNAs were gradually increased during the culture. Under non-stressed culture condition, the hydrogel was compacted by cell-mediated contraction, released from culture dish, and finally formed a stratified cell sheet. This self-compacted stratified cell sheet was easily manipulable and grafted on the epicardium of acute infarcted heart. CSCs populated in a stratified sheet transepicardially migrated into the injured myocardium and participated the myocardial regeneration. Overall, our unique approach may be broadly applicable to fabricate a stratified CSC sheet for the myocardial regeneration.

W-3144

CELL-SHEET-BASED BIOENGINEERED HUMAN CARDIAC TISSUE USING PLURIPOTENT STEM CELLS FOR HEART REPAIR AND DISEASE MODELS

Katsuhisa Matsuura[1], Tatsuya Shimizu[1], Yuji Haraguchi[1], Masanori Wada[3], Nobuhisa Hagiwara[2], Teruo Okano[1]
[A]Institute of Advanced Biomedical Engineering and Science, [B]Department of Cardiology, [1]Tokyo Women's Medical University, Tokyo, Japan, [2]I, ABLE CO., Tokyo, Japan

We have developed an original and unique scaffold-free tissue engineering approach, “cell sheet engineering”, using a temperature responsive culture surface, and this technology has been already applied to regenerative medicine of various tissues such as cornea, heart and esophagus in the clinical setting. As the bioengineered three-dimensional cardiac tissue is expected to not only function for repairing the broad injured heart but also to be the practicable heart tissue models for drug screening and the disease models, we have developed the cell sheet-based bioengineered vascularized three-dimensional cardiac tissue. Furthermore the development of human iPS cell technology enables us to create human cardiac tissue. Recently we have developed the novel suspension cultivation system for undifferentiated expansion and the high-efficient cardiac differentiation of human iPS cells. This unique stirring system with the suitable dissolved oxygen concentration was capable of expanding human iPS cells with maintaining the undifferentiated state up to 1×10^8 cells/100 ml in 5 days. Moreover fourteen-day culture with the serial treatments of suitable growth factors and a small compound produced robust embryoid bodies that showed the spontaneous beating and the cell number was increased to around 8×10^7 cells/100 ml vessel. Flow cytometric analysis revealed that about 80% and 5% of cells were cardiac troponin T positive cardiomyocytes and CD31 positive endothelial cells, respectively. When cells after cardiac differentiation and the enzymatic dissociation were cultured on temperature-responsive culture dishes, the spontaneous and synchronous beating was observed accompanied with the intracellular calcium influx all over the area even after cell sheets were detached from culture dishes by the lowering the culture temperature. High content image analysis revealed that the cardiac cell sheets were mainly composed of cardiomyocytes (80%) and partially mural cells (20%). Consistent with the results that the mRNA expression of pluripotency genes such as Oct3/4 and Nanog were further diminished after the process of fabricating cardiac ell sheets, undifferentiated cells such as Oct3/4 expressing cells were not observed. Furthermore, extracellular action potential propagation was observed between cell sheets when two cardiac cell sheets were partially overlaid, and this propagation was inhibited by the treatment with some anti-arrhythmic drugs. When the triple layered cardiac tissue was transplanted onto the subcutaneous tissue of nude rat, the spon-

taneous pulsation was observed at 2 weeks and engrafted cardiomyocytes were vascularized with the host tissue-derived endothelial cells. These findings suggest that cardiac cell sheets formed by hiPSC-derived cardiomyocytes might have sufficient properties for the creation of thickened cardiac tissue. Now we are developing the vascularized thickened human cardiac tissue by the repeated layering of cardiac cell sheets on the artificial vascular bed in vitro. In this session, I am going to show our recent progress of cell-sheet-based cardiac tissue engineering for heart tissue repair and as possible applications for human heart disease models.

W-3145

CARDIOMYOCYTE DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS IN CHEMICALLY DEFINED E8 MEDIA

Yongshun Lin, Kaari Linask, Wen Xie, Yanjun Qin, Jeanette Beers, Guokai Chen
National Heart, Lung, and Blood Institute, Bethesda, MD, United States

Because of their unique capacity to generate any cell type of the human body, human pluripotent stem cells (hPSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), hold great promise for drug discovery and cell therapy. To provide an effective tool to facilitate research use and satisfy the clinical demands of hPSCs, we previously developed a simplified chemically defined media (E8) for hPSCs derivation and culture, which is completely free of human and animal products including serum and albumin. In this study we developed a protocol for the differentiation of clinical-grade cardiomyocytes from hPSCs on the defined E8 medium platform. By modulation of exogenous and endogenous recombinant cytokines and hormones, such as Wnt, BMP, and Activin, as well as the components (TGFb, FGF2, and insulin) of E8, we demonstrate that beating cardiomyocytes can be efficiently induced from hPSCs (ESCs and iPSCs) using E8 based medium. E8 can be successfully applied in both 2D monolayer and 3D embryoid bod (EB) culture. Since the whole procedure is under fully defined conditions, our approach provides a promising and powerful tool for generating desired cardiac lineages that could be utilized in basic research and clinical transition study.

W-3146

Improving cardiomyogenic differentiation of human pluripotent stem cells (hPSCs) in defined conditions by the application of small molecules

Kepf Henning, Ruth Olmer, Gerald Dräger, Ulrich Martin, **Robert Zweigerdt**
REBIRTH - Center for Regenerative Medicine, Hannover, Germany

Improving cardiomyogenic differentiation of human pluripotent stem cells (hPSCs) in defined conditions by the application of small molecules
Estimations suggest that more than one billion cardiomyocytes per patient will be required for cardiac replacement therapies after myocardial infarction. Human pluripotent stem cells (hPSC) present an attractive cell source to generate these large amounts, not only for the envisioned cell therapies, but also for drug screening and cardiotoxicity tests.
For these purposes an efficient hPSC differentiation process is highly demanded, preferentially in defined and scalable conditions. To this end we and others have successfully developed scalable expansion culture of hPSCs in suspension culture.
However, efficient cardiomyogenic differentiation strategies for these cultures were not described yet. Towards this end we have tested chemical compounds including p38 MAPK- and Wnt- pathway modulators alone and in combination in multi well assays and monitored cardiac differentiation by an Nkx2.5-eGFP reporter line, KDR/PDGFR-α FACS, and cardiac Troponin T-specific immunoflourescence staining. Promising candidate combinations were subjected to hPSC mass suspension cultures. In this context we tested a) different culture platforms particularly focusing of chemically defined culture media and b) tested several hPS cell lines to show general applicability and robustness of our novel conditions.

We have achieved an efficient hPSC differentiation process i.e. observed a level of >60% CMs induction based on small molecules enabling the derivation of large amounts of functional cardiomyocytes in chemically defined conditions.

W-3147

TRIIODOTHYRONINE TREATMENT PROMOTES THE MATURATION OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

Xiulan Yang, Veronica Muskheli, Mark Saiget, Lil Pabon, Hans Reinecke, Charles E. Murry
University of Washington, Seattle, WA, United States

Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) hold great promise for regeneration of diseased hearts. There is, however, a risk of arrhythmias following hPSC-CMs transplantation into injured hearts. Enhancing their maturation may reduce the chance of arrhythmia after transplantation. Tri-iodo-L-thyronine (T3) is reported to be a prime driver of sheep cardiomyocyte maturation. In this study, we tested the hypothesis that T3 treatment of hPSC-CMs would result in the maturation of their structural and contractile properties to a more adult-like phenotype. Compared to control cells, T3-treated cells showed significant differences in morphology, including increased cell size and anisotrophy, increase in the percentage of cardiomyocytes with multinucleation, as well as an increase in sarcomere length. We measured the contractile performance of these cells and noted a significant increase of departing velocity. Q-PCR showed that the T3-treated cells have a higher alpha-myosin heavy chain, sarcoplasmic reticulum Ca2+ ATPase, and a lower level of beta-myosin heavy chain and Connexin 40. Altogether, these findings indicate that T3 enhances the maturation of human pluripotent stem cell derived cardiomyocytes.

W-3148

MODELING HYPERTROPHIC CARDIOMYOPATHY USING HUMAN IPS CELL DERIVED CARDIOMYOCYTES

MOHAMMAD A. MANDEGAR, JENNIE YOO, LUKE M. JUDGE, TRIEU NGUYEN, JUAN A. PEREZ-BERMEJO, YUICHIRO MIYAOKA, PAWEENA P. LIZARRAGA, AMANDA CHAN, NATHANIEL HUEBSCH, MATTHEW J. SPINDLER, IAN SPENCER, PO-LIN SO, BRUCE R. CONKLIN
Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, United States

Hypertrophic cardiomyopathy (HCM) is the most common inherited form of cardiovascular disease. It is the leading cause of sudden cardiac death in young athletes, and its prevalence has been estimated to be as high as 1 in 500 within the general population. The majority of HCM-associated mutations have been identified in sarcomeric proteins, in particular the cardiac isoform of Myosin Binding Protein C (*MYBPC3*). Our research is focused on elucidating the molecular basis for how a common 25bp deletion in intron 32 of *MYBPC3* (*MYBPC3*$^{\Delta 25}$) might lead to the development of HCM. Despite the high prevalence of this mutation - with an estimated 50 million carriers worldwide - remarkably little is known about the molecular mechanisms that may lead to the development of HCM. The current hypothesis posits that this mutation causes significant structural modifications at the MYBPC3 C-terminus that alters its binding affinity for sarcomeric proteins involved in regulating actomyosin sliding filament motion (e.g., Titin and LMM-Myosin), and these changes ultimately lead to the development of HCM phenotypes. However, investigating the relationship between *MYBPC3*$^{\Delta 25}$ deletion and the HCM phenotype has been hampered by the lack of adequate cellular models. Although some in vitro models of HCM have been created with human induced pluripotent stem (iPS) cells, none have yet been made for *MYBPC3*.
To overcome these challenges, we are using Transcription Activator-Like Effector Nuclease (TALEN)-directed homologous recombination to genetically engineer homozygous and heterozygous knockouts (KO) of *MYBPC3* and the *MYBPC3*$^{\Delta 25/\Delta 25}$ variant into isogenic iPS cell lines. We have genetically engineered both heterozygous and homozygous *MYBPC3* KO iPS cell lines by inserting an artificial early stop codon into exon 1 of *MYBPC3*. Preliminary results suggest a differential beat rate phenotype in the heterozygous KO iPS-CM compared to WT control iPS-CM, and delayed cardiomyocyte maturation in homozygous KO iPS-CM compared to WT control and heterozygous KO lines. We are currently in the process of determining robust cellular phenotypes using electrophysiology and cellu-

lar morphometric assays. In addition, we are engineering a FLAG epitope tag to reveal key binding partners of the 'wild type' and mutant forms of MYBPC3 via immunoprecipitation from iPS-derived cardiac myocytes. These lines will be used in comparative phenotypic and proteomic analyses to yield critical insight into the molecular mechanisms of this disease. We expect these studies will improve our understanding of the pathological mechanisms underlying HCM and could lead to the identification of drug targets and enhanced treatment options for HCM patients.

W-3151

DRIVING IN VIVO HEART PROGENITOR CELL FATE AND VASCULOGENESIS VIA CHEMICALLY MODIFIED MRNA

Lior Zangi[1], Kathy Lui[3], Alexander von Gise[1], Derrick Rossi[2], William Pu[1], Kenneth Chien[4]
[A]Cardiology, [B]Cellular and Molecular Medicine, [1]Boston Children's hospital, Harvard medical school., Boston, MA, United States, [2]Department of Stem Cell and Regenerative Biology, Harvard University, Boston, MA, United States, [3]Department of Cell and Molecular Biology and Medicine, Karolinska Institutet, Boston, Sweden

Translation of our growing knowledge of molecular pathogenesis into effective therapy has been hampered by limitations of available gene transfer platforms. RNAs containing chemically modified nucleotides ("modRNAs") have been shown to be non-toxic, non-immunogenic, and efficiently translated into functional proteins. Here, we show that modRNAs are a novel and efficient gene transfer platform for heart and skeletal muscle. We reasoned that the "pulse-like" expression kinetics of modRNAs might be used to express paracrine factors as cell fate switches to redirect progenitor cell fate and thereby enhance long term organ repair. As proof of concept, we used modRNA to drive high level expression of human vascular endothelial growth factor-A (VEGF-A) in the heart. Administered in the setting of myocardial infarction (MI), VEGF-A modRNA markedly improved heart function and enhanced long-term survival of recipients. This improvement was largely due to reduced infarct size and increased capillary density in the peri-infarct myocardium. Enhanced myocardial vascularization was in part due to a novel activity of VEGF-A on Wilm's tumor 1 (Wt1)-expressing epicardial heart progenitors: VEGF-A mobilized and augmented this heart progenitor pool, and redirected their differentiation towards vascular cells. Our results establish a novel therapeutic paradigm in which transient application of paracrine factors alters progenitor cell behavior and fate to achieve sustained improvement of therapeutic endpoints. modRNA represents a new, highly translatable, cell-free approach to implement this therapeutic paradigm and enhance repair of the heart, vasculature and potentially other solid organs.

W-3152

ISOLATION OF CARDIAC PROGENITORS FROM MURINE AND SIMIAN POST MORTEM CARDIAC TISSUE

Daisuke Kami, Midori Nakata, Tomoya Kitani, Satoshi Gojo
Kyoto Prefectural University of Medicine, Kyoto, Japan

Background
Cell-based regeneration therapies hold great promise and potential for new area in clinical medicine, although some obstacles still remain to be overcome for a wide range of clinical applications. Currently, the cells for transplantation were derived from either autologous or allogeneic tissue. The former has a drawback that the quality of donor cells could depend on the patient's condition, and the quantity could also be limited. A phase I clinical trial

named ALCADIA (ClinicalTrials.gov Identifier: NCT00981006), which stand for AutoLogous human CArdiac-Derived stem cell to treat Ischemic cArdiomyopathy, has been completed. Hereof, it has taken several weeks to grow cardiac progenitors up to acquire the cell number to be needed for the treatment. To solve these problems, we are investigating the potential of allogeneic cardiac progenitors transplantation derived from post mortem human heart.
Objective
To examine whether cardiac progenitors could be isolated from murine and simian post mortem cardiac tissue.
Methods
Cardiac progenitors were isolated from the right and left atrial appendage and free wall of bilateral ventricles of mice (10-18 weeks old, C57BL/6), and cynomologus monkey (5 to 9 years old, Macaca fascicularis). Cardiac tissue was harvested either at just sacrifice or 24 hours post mortem. The isolation of cardiac progenitors was carried out according to the current protocol for ALCADIA. The cardiac progenitors were cultured in a DMEM/F12 supplemented with 10% FBS and 40 ng/ml of basic FGF onto 60-mm Collagen I coated culture dish. At two to three passages of cardiac progenitors, we performed the cellular characteristics analysis, which included growth rate, RT-PCR, FACS, and microarray analysis.
Results
Cardiac progenitors could be isolated from both murine and simian heart 24 hours post mortem. Compared with cardiac progenitors from the heart to be harvested just after sacrifice, post mortem cardiac progenitors had longer lag phase after seeding, and they demonstrated the similar characters in respect of the surface antigens and transcriptional gene expression. FACS analysis showed cardiac progenitors expressed CD29, CD90, and CD105, and were negative for CD31, CD45, and CD117. RT-PCR demonstrated that cardiac progenitors were positive for Gata4, Tbx5, and Nkx2.5 as markers of early cardiac development, and Klf4 and cMyc as markers of pluripotency. Otherwise, Oct4, Sox2, and Nanog as markers of pluripotency, and Brachyury, and Mesp1 as markers of mesodermal stem cells, and c-kit as a marker of somatic stem cells were negative in both fresh and post mortem population.
Imprications
These results indicate allogeneic cardiac progenitor cell transplantation using post mortem heart feasible. This strategy could easily transfer to off-the-shelf product model in pharmaceutical company.

W-3153

INCREASE OF CAPILLARY DENSITY DURING PREGNANCY IN MOUSE IS CAUSED BY HORMONES

Daniel Korzus[1], Kristina Thaben[1], Stuart Walsh[2], Stefan Jovinge[3], Bernd Kurt Fleischmann[1], Michael Hesse[1]
[1]Institute of Physiology I, University of Bonn, Bonn, Germany, [2]Department of Cardiology, Children's Hospital Boston, Boston, MA, United States, [3]Lund Strategic Research Center for Stem Cell Biology and Cell Therapy, Lund University, Lund, Sweden

During pregnancy the organism undergoes a variety of adaptive physiological changes. The cardiovascular system is strongly affected and its response characterized by the hypertrophy of the heart. The molecular mechanisms underlying these adaptations are poorly understood. Because of the recently developed concept of resident organotypic stem cells in the cardiovascular system, our aim is to explore the potential involvement of these cells and the related signaling cascades in this adaptation process in the heart.
We have analyzed cell proliferation using immunostainings against pHH3/Ki67 in mouse hearts at different stages during pregnancy. The average percentage of Ki67+ nuclei per total nuclei in cardiac sections increased from virgin controls to gestational day 3 (GD3) and peaked at GD14. Enhanced proliferation immediately stopped after delivery. Co-staining revealed that approximately 2/3 of the proliferating cells were fibroblasts and 1/3 endothelial cells (ECs). The lack of cardiomyocyte (CM) proliferation was corroborated using BrdU pulse chase experiments examined by Langendorff perfusion. The results were confirmed by flow cytometric analysis. The proliferation of fibroblasts and ECs reflects angiogenesis, as could be measured by an increase of capillary density, and extracellular matrix remodeling known to occur during pregnancy induced hypertrophy. To analyze the role of hormones in these physiological adaptive processes, plasma levels of progesterone, prolactin and estrogen were quantified. Progesterone- and prolactin- levels showed their maximal blood concentrations at GD14 and declined at the day of

delivery coinciding with the course of proliferation, whereas estrogen showed two distinct peaks at GD3 and GD18. To mimic the action of pregnancy hormones we implanted hormone-pellets with consistent release for 21 days in ovariectomized mice subcutaneously. Capillary density was significantly increased in mice receiving progesterone-pellets alone or in combination with estrogen-pellets without induction of hypertrophy. Thus, increase of capillary density is directly caused by pregnancy hormones.
We are currently investigating the mechanisms underlying pregnancy hormones induced proliferation in cardiac ECs and fibroblasts.

W-3154

BIOLOGICAL PACEMAKER DERIVED FROM PLURIPOTENT STEM CELLS FOR TREATMENT OF BRADYCARDIA

Kumi Morikawa[1], Yoshinori Yoshida[2], Yasuaki Shirayoshi[1], Ichiro Hisatome[1]
[1]Department of Regenerative Medicine and Therapeutics, Graduate School of Medical Science, Tottori University, Yonago, Japan, [2]Department of Reprogramming Science, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

An inward current (designated I_f) activates the membrane hyperpolarization in cardiac pacemaker cells at sinoatrial node (SAN), and is responsible for spontaneous pacemaker activity and rate control. Of the four hyperpolarization-activated cyclic nucleotide-gated (HCN) ion-channel family genes, which contribute to native I_f current, HCN4 is the dominant isoform in cardiac pacemaker cells. Although the progress of biological pacemakers has been made with viral vectors, human embryonic stem cells, and adult human mesenchymal stem cells as delivery systems, no approach to biological pacemaking has been to utilize the HCN4 gene or HCN4 positive cardiac pacemaker cells. Cardiac pacemaker cells can be extracted from the differentiating mouse embryonic stem (mES) cells on the basis of their specific expression of ion-channel HCN4 at SAN. We have established the green fluorescent protein (GFP) knock-in mESCs at HCN4 locus (H7 clone) and characterized pace-making cells derived from H7 ES cells by isolation of GFP-positive cells using embryoid body (EB) differentiating system.
The expression of GFP was specifically restricted at their contracting region in differentiating H7 EBs. Cell sorting revealed that a few cells (0.1~0.5%) of contracting H7 EBs were GFP positive. Approximately 80% of GFP+ cells showed the spontaneous beating activity, which was based on cesium sensitive action potentials. Sorted GFP+ cells expressed endogenous HCN4 (HCN4+) and had essentially the same properties as the cardiac pacemaker cells at SAN. At first, GFP+ cells had the common gene and protein expression profiles with cardiac pacemaker cells, for examples, pacemaker specific makers such as HCN4, Cav3.1 and Connexin43 as well as cardiomyocyte specific maker, Tropomyosin C. Second, I_f currents were confirmed by patch-clump analysis. Finally, GFP+ cells were capable of actively responding to adrenergic stimulation and cholinergic repression.
We further investigated whether mESC-derived HCN4+ cells can restore myocardial electromechanical properties. Using imaging techniques, we demonstrated that HCN4+ cells established electrical coupling with HL-1 cells, a cardiac muscle cell line derived from the mouse atrial cardiomyocytes tumor, to induce rhythmic electrical and contractile activities *in vitro*. Similarly, the transplanted HCN4+ cells paced the hearts of rat with complete atrioventricular block, indicated that HCN4+ cells could substitute for pacemaker cells and elicit an ectopic rhythm *in vivo*.
These results demonstrated the potential of mESC-derived HCN4+ pace-making cells to act as a rate-responsive biological pacemaker and for future myocardial regenerative medicine to bradycardia.

W-3155

ALLOGENICITY OF HUMAN CARDIAC STEM/PROGENITOR CELLS

Laura Lauden, Wahid Boukouaci, Noemie Dam, Ryad Tamouza, Dominique Charron, **Reem Al-Daccak**
UMRS940, INSERM, Paris, France

Rationale: Transplantation of allogeneic cardiac stem/progenitor cells (CPC) in experimental myocardial infarction promoted cardiac regeneration and improved heart function. While this has enhanced prospects of using allogeneic CPC for cardiac repair, the mechanisms regulating the behavior of these allogeneic cells, which are central to clinical applications, remain poorly understood.
Objective: T cells orchestrate the allogeneic adaptive immune response whereas natural killer (NK) cells are major players of innate immune response. Therefore, to provide insight into the mechanisms regulating the immunologic behavior of human CPC (hCPC), we investigated the T and NK cells response to allogeneic hCPC.
Results: We demonstrate that, whether under inflammatory conditions or not, hCPC do not trigger conventional allogeneic Th1 or Th2 type responses but instead promote a regulatory response and an allogeneic-driven immunomodulation, both dependent on B7 family member programmed death-ligand 1 (PD-L1). Activated NK cells were able to lyse allogeneic hCPC, but under inflammatory conditions a drastic decrease in this killing was observed. hCPC were also able to modulate the NK cytolytic activity towards target cells and this was accompanied by a decrease in the expression of NK activating receptors. Although, the mechanisms underlying the behavior of NK cells against allogeneic hCPC are still under investigation, our data indicate that hCPC are probably protected against NK cell lysis upon their application in an inflammatory context as after myocardial infarction.
Conclusions: Collectively our data reveal that hCPC in allogeneic settings have a “tolerogenic” immune behavior. Our study attributes an important role for PD-L1 and raises the possibility of using PD-L1 expression as a marker to identify and select low-risk high-benefit allogeneic cardiac repair cells.

W-3156

SILENCED ALPHA ADRENERGIC SIGNALLING INHIBITS HYPERTROPHY OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

Gabor Foldes[1], Elena Matsa[2], Janos Kriston-Vizi[3], Thomas Leja[1], Stefan Amisten[4], Ljudmila Kolker[1], Maxime Mioulane[1], Karine Vauchez[1], Tamas Aranyi[5], Robin Ketteler[3], Michael Schneider[1], Chris Denning[2], Sian Harding[1]
[1]National Heart and Lung Institute, Imperial College London, London, United Kingdom, [2]University of Nottingham, Nottingham, United Kingdom, [3]University College London, London, United Kingdom, [4]Oxford University, Oxford, United Kingdom, [5]Université d'Evry, Evry, France

The potential of stem cell-based disease modelling is enhanced by the realisation that cardiomyocytes from human embryonic stem cells (hESC-CM) and induced pluripotent stem cells (hiPSC-CM) can be obtained with disease-specificity. Hypertrophy is a high priority target because of its central role in the transition to heart failure. Strikingly, here we found that hiPSC-CM are relatively unresponsive to major hypertrophic signals compared to hESC-CM. We show that the normal alpha-adrenergic receptor 1A subtype (ADRA1A) is not expressed robustly in either cell type. ADRA1A is reversibly silenced during differentiation, accompanied by up-regulation of ADRA1B, resulting in a distinct gene profile from that in adult human cardiomyocytes. Loss of ADRA1A is more pronounced in hiPSC-CM, due to greater epigenetic silencing and more marked up-regulation of HIF-1α, but ultimately both cell types differ from adult in their reliance on active ADRA1B rather than ADRA1A. ADRA1B up-regulation is sufficient in hESC-CM for hypertrophic changes such as cell size, cell volume and ANF. However, in hiPSC-CM, additional decreased G-protein signalling and tonically inhibitory pathway networks suppress the effect of alpha-adrenoceptor stimulation on growth. Superficial similarities between hESC-CM, hiPSC-CM and adult cardiomyocytes may mask complex differences in signalling. These data raise serious questions regarding the hiPSC-CM as a valid model system for certain aspects of cardiac disease.

Eye or Retinal Cells

W-3161

CELL ASSORTMENT IN ORGAN DEVELOPMENT

Philip Iannaccone[1], Jerry Rhee[1], Steve Iannaccone[1], Talisa Mohammad Nejad[2], Craig Foster[2]

[1]*Pediatrics and Pathology, Northwestern Uninversity, Lurie Children's Research Center, Chicago, IL, United States,* [2]*Dept. of Materials Science and Engineering, University of Illinois in Chicago, Chicago, IL, United States*

Following allocation of stem cells to organ primordia parenchymal cells assort themselves in a robust way allowing further functional organization. The correct placement of cells is more complex than can be managed by direct genetic control. A more likely explanation is self-organizing algorithmic growth. This mechanism has been established as an explanation for the patterns of cell allocation made visible by analysis of mosaic pattern in animals comprising multiple distinguishable lineages, for example in chimeras made by amalgamating embryos of strains differing in RT1 genes or in the expression of reporter genes such as GFP. In the liver, cells are ultimately arrayed without regard to major anatomical features. In the adrenal cortex on the other hand cortical cells form parallel arrays that align with major vessels. Division rule sets that function as cellular automata operating on stem cell pools have been established and are sufficient to explain the dominant patterns observed. In the developing cornea patterns are observed in mouse and rat that like the liver are seemingly unstructured, like islands of one cell lineage in a sea of the other. But within weeks in the mouse and months in the rat the pattern changes to spiral arrays of epithelial cells. The spirals are logarithmic and can be clockwise or counterclockwise with contralateral similarity. Cell division rates, and patterns that double back to create saddle structures belie a simple explanation based on the division of stem cells in the limbal area of the developing cornea. We developed an alternative hypothesis; that shear strain facilitates cell sliding. Shear strain is a result of intraocular pressure, corneal shape and collagen fiber orientation in the stroma. These factors were modeled using sophisticated numerical methods with finite elements analysis. Spiral patterns correlated to directions of maximum shear strain indicating that shear strains on the anterior surface of the stroma may facilitate movement of the epithelial cells into spirals as they slide past each other. The directional stiffness imparted by the collagen fibrils moderately influences the local curvature of the spirals, and hence the local arrangements may be important to the formation of these spiral patterns. While the resulting shear strains from our model do form spirals that are close to logarithmic, the pitch angle is much higher than for observed spirals. Hence shear strain alone is not an adequate explanation for the motion. It could be that that dynamic changes in shear strain coupled with migration and perhaps other phenomena could explain the spiral formation, but further investigation is necessary to fully understand the underlying causes of the spiral generation. Stem cell dynamics and cell assortment are useful probes of processes important in organogenesis.

W-3162

DEVELOPMENT OF AN APPROACH FOR IN VITRO GENERATION OF RETINAL GANGLION-LIKE CELLS BY DIRECT REPROGRAMMING

Valentin Sluch[1], Donald J. Zack[2]
[A]*BCMB,* [B]*Ophthalmology,* [1]*Johns Hopkins University School of Medicine, Baltimore, MD, United States*

The optic nerve is composed of retinal ganglion cell (RGC) axons that relay visual information from the eye to the brain. A number of optic nerve diseases lead to RGC death and vision loss, the most common of which is glaucoma, the second leading cause of blindness in the world. Rodent models have proved useful for the study of optic nerve disease. However, these models represent an approximation of the human condition, and may not replicate all the necessary molecular events involved. Generation of human RGCs in vitro, through either stem cell or direct reprogramming methods, could make possible an approach for medically relevant drug screening that would have advantages over the use of rodent cells. In addition, such cells could provide insights into human RGC development, gene regulation, and general biology.
Unfortunately, stem cell derivation of RGC-like cells is inefficient and time consuming. In an effort to circumvent these challenges, we have been working to develop a direct reprogramming method to convert somatic cells directly into RGCs. Through lentivirus induced overexpression of a set of transcription factors relevant to RGC biology, we were able to transdifferentiate human retinal pigment epithelium cell line cells (ARPE19) to cells with some characteristics of the RGC lineage over a course of three weeks. These induced RGC-like cells (iRGC) exited the cell cycle and exhibited a dendritic arborization pattern reminiscent of RGCs. Immunocytochemistry showed that iRGCs

expressed neuronal markers TUJ1 and MAP2 and expressed the RGC markers CALB2 and BRN3A. qPCR analysis showed upregulation of expression of the following RGC enriched genes - SNCG, THY1, and NHLH2 as compared to negative controls and a transcription factor set for generation of cortical neurons.
In conclusion, we have made progress in generating human RGC-like cells by direct reprogramming of an established retinal pigment epithelium cell line. Using the identified genes necessary for RGC cell fate determination in this system, we hope to develop methodology to convert other cells types such as fibroblasts to RGC-like cells, as well as illuminate important factors for RGC competence. Furthermore, these iRGC cells should provide a valuable resource for drug screening and studying reprogramming mechanisms as well as RGC biology.

W-3163

MODELING HUMAN CONE PHOTORECEPTORS DEVELOPMENT AND DEGENERATION USING EMBRYONIC STEM CELLS

Gilbert Bernier
Research Center, Hôpital Maisonneuve-Rosemont, Montreal, QC, Canada

Inherited juvenile macular degeneration, dry age-related macular degeneration and cone dystrophies represent a major cause of blindness. These pathologies primarily lead to degeneration of cone photoreceptors. Cones are involved in color discrimination and high acuity central vision. However, current source of human cones to model photoreceptor development and disease in vitro does not exist. We report here on the rapid and efficient differentiation of human embryonic stem cells into cone photoreceptors. These cells are amenable to recapitulate the developmental genetics of human photoreceptors and to model cone diseases. We provide one such example by knockdown of the Polycomb group gene BMI1 using shRNA-producing lentivirus, which leads to progressive cone degeneration in vitro.

W-3164

COMBINED CELL AND GENE THERAPY TOWARDS THE TREATMENT OF AGE-RELATED MACULAR DEGENERATION AND DIABETIC RETINOPATHY

Sabiha Hacibekiroglu[1], Iacovos P. Michael[1], Peter Westenskow[2], Brian Ballios[3], Derek van der Kooy[3], Martin Friedlander[2], Andras Nagy[1]
[1]Samuel Lunenfeld Research Institute/Mount Sinai Hospital, Toronto, ON, Canada, [2]The Scripps Research Institute, San Diego, CA, United States, [3]Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada

Age-related macular degeneration (AMD) and diabetic retinopathy (DR) are the leading blindness causing eye diseases that affect more than 14 million people worldwide. Both are associated with abnormal and leaky blood vessel growth (neovascularization) caused by an up-regulation of vessel endothelial growth factor (VEGF-A) that leads to vascular leakage, vessel dilatation, tortuosity, haemorrhage and ultimately to cell death and blindness. Current treatments include injection of anti-angiogenics such as anti-VEGF-A antibody (e.g. Ranibizumab). Such treatments improve visual function through regression of abnormal blood vessels. However, patients require monthly injections due to its transient effect, and these treatments cause side effects such as stroke, gastrointestinal perforations and bleeding. We hypothesize that a combination of cell and gene therapy can inhibit abnormal blood vessel growth in eye diseases such as AMD and DR in a controlled and long-term manner, thereby improving vision and diminishing any potential side effects.
The aim of this study is to generate transgenic cells that secrete locally acting anti-VEGF biologics for minimally invasive and controlled long-term administration. We have generated a novel bi-functional VEGF sticky-trap (by modifying the original VEGF trap) that is able to trap VEGF as well as bind (i.e. "stick") to the extracellular matrix (ECM) through binding to heparan-sulphate proteoglycans. This consequently allows inhibition of neovascularization only at the site of expression or administration. We have shown that VEGF sticky-trap, upon intravitreal and subretinal injection, binds to the ECM components of the eye. In contrast to the original VEGF trap, VEGF sticky-trap was un-

detectable in circulation 6 hrs post eye injection. These results indicate that VEGF Sticky-trap remains locally at the site of administration and once it enters the circulation it is degraded within a short amount of time (<8hrs) limiting systemic side effects. Furthermore, we have shown that VEGF sticky-trap is able to inhibit neovascularization in a murine model of DR demonstrating the effectiveness of this biologic in an in vivo model.
Functional retinal pigment epithelium (RPE) cells and mesenchymal stem cells expressing VEGF sticky-traps in a doxycycline-inducible manner have been generated. We have shown that VEGF sticky-trap, expressed by these cells in vitro, is able to bind to cell ECM and trap soluble VEGF only upon doxycycline induction demonstrating a controlled production of VEGF sticky-trap. In addition, we have characterized the ability of the transgenic RPE cells to incorporate into the eye. Currently, we are evaluating the ability of these cells to express VEGF sticky-trap in vivo and to inhibit NV and improve vision in animal models of AMD, such as the RCS rat and the laser-induced chorodial NV mouse.
The injection of these cells into the diseased eye will allow for long-term treatment of AMD and DR by replacing atrophic cells for photoreceptor maintenance, inhibition of neovascularization and regression of abnormal blood vessels controlled at all times by drug (doxycycline, DOX) administration (ON/OFF system). Ultimately, this novel approach may translate into improved treatment options for patients with AMD and DR and contribute to currently on-going clinical trials using RPE cell therapy in patients with AMD.

W-3165

HUMAN ESC-DERIVED CORNEAL ENDOTHELIAL CELLS FOR THE TREATMENT OF CORNEA DYSFUNCTION.

Soyeon Kwon, Yinyin Chen, Guoping Fan
UCLA, Los Angeles, CA, United States

Cornea endothelial cells (CECs) consist of monolayer cells in hexagonal shape, neighboring each other by tight junction. CEC deficiency due to genetic disorder, trauma, and aging would cause serious corneal edema and blindness in patients. Since adult CECs do not have any capacity to proliferate and self-renew *in vivo,* CEC transplantation is the only treatment. In this study, we attempt to produce CECs from human embryonic stem cells (hESCs). H9 hESCs were first differentiated into neural crest stem cells (NCSCs) by Noggin and SB431542, and NCSCs were affinity-purified via antibody against neural crest marker, HNK-1. The purified NCSCs were cultured with conditioned medium from primary CEC cultures. Approximately 1-2 weeks in culture, we observed a subset of cells exhibiting the polygon morphology of CEC-like cells. We are currently characterizing these CEC-like cells by examining gene expression profiles with a focus on CEC-related markers such as COL8A1, Na^+/K^+-ATPase and Aquaporin1 (AQP-1). We will present our new data on functional characterization of these hESC-derived CECs at the meeting. Our approach may provide a valuable and unlimited source of human CECs for future clinical treatment of CEC deficient disorders.

W-3166

EFFICIENT DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO RETINAL CELL TYPES

Indumathi Mariappan, Savitri Maddileti, Jamila H. Siamwala
SS Ravi Stem Cell Laboratory, Hyderabad Eye Research Foundation, LV Prasad Eye Institute, Hyderabad, India

Purpose: To establish an efficient protocol to derive retinal cell types from a well characterized Indian human embryonic stem cell line, BJNhem20 (UKSCB accession No. R-08-022).
Methods: The human embryonic stem cell line, BJNhem20 (Inamdar et al., 2009), was cultured and expanded in a feeder-free culture system. Differentiation was initiated by embryoid body (EB) formation in suspension cultures on non-adherent plates with ES medium devoid of bFGF (differentiating medium, DM). After 3 days, the EBs are grown as adherent cultures in neural induction medium (NIM) (DM + 1% N2 supplement) on laminin coated dishes for 3 days. The colonies showing neural rosettes were isolated and further cultured as suspended neurospheres in retinal differentiation medium (RDM) (NIM + 2% B27 supplement) for 3 days. The neurospheres were further plated on laminin coated dishes and maintained as adherent cultures in RDM for about 3 months. The differentiated cells were characterized for retina-specific marker expression by immunofluorescence and RT-PCR analysis.

Results: Using the differentiation protocol described, we could efficiently derive different retinal cell types from the human ES cell line, BJNhem20. Cells expressing retinal progenitor markers (Nestin, Pax6, Chx10, Rx, Otx2, Mitf) and retinal pigmented epithelial (RPE) cells with cobble-stone morphology are observed after 3-4 weeks of differentiation. These early progenitors were amenable for further expansion and enrichment. The mature retinal cells expressed several neural retina-specific markers such as β-III Tubulin, GFAP, MAP2, Rhodopsin, PDE6a, PDE6b and RPE-specific markers such as ZO-1, Tyrosinase, Bestrophin, Mertk, RPE65 and acquired pigmentation in long-term cultures.
Conclusion: The BJNhem20 cells can be efficiently differentiated into neuro-retinal and RPE cells using the differentiation protocol described here.
Key words: Human embryonic stem cells, retinal pigmented epithelial (RPE) cells, retinal dystrophy.

W-3167

TRANSPLANTATION OF HUMAN RETINAL PROGENITOR CELLS IN RCS RAT MODEL OF RETINAL DEGENERATION: RESCUE OF HOST RETINAL STRUCTURE AND FUNCTION

Jing Luo[1], Peter Shaw[1], Petr Baranov[2], Gary Brooke[3], Caroline Hicks[3], Jessica Lu[1], Hong Ouyang[1], Jiaogang zhao[1], Sara Patel[3], Victoria Reade[3], John Sinden[3], Michael Young[2], Kang Zhang[1]
[1]Department of ophthalmology, Shiely Eye Center, UCSD, San Diego, CA, United States, [2]Schepens Eye Research Institute, Massachusetts Eye and Ear, Boston, MA, United States, [3]Reneuron, Guildford, United Kingdom

PURPOSE: To investigate the therapeutic potential of two human Retinal Progenitor Cells (hRPC) cell lines transplanted into subretinal space of Royal College of Surgeons (RCS) rats to rescue host retinal structure and function.
METHODS: Fetal eye tissue was procured in compliance with IRB and GTP guidelines. 18 week fetal retinal progenitor cells were cultured and expanded to passage 8 and frozen to provide banks of hRPCs. Cells were revived from P8 banks and grown to P9, and transplanted into subretinal space of RCS rats at 2.5x104 cells/µl. RCS rats were injected in the right eye (OD) with A) hRPC line A; B) hRPC line B; C) vehicle only (V, HBSS-NAC); D) untreated controls and compared to baseline control without injection. Group A and B were injected subretinally with 2 µl of cells. Animals were immunosuppressed with oral Cyclosporine A throughout study and IP dexamethasone for 14 days post-transplant. Visual acuity (VA) tests with optokinetic nystagmus (OKN) were conducted at 5 and 12 weeks post-transplant. At 12 week eyes were harvested, and retinal histology assessed.
RESULTS: VA measurement showed that rats treated with hPRC line A (OS vs OD: 0.38 vs 0.46, c/d, $p < 0.05$ or V (OS vs OD: 0.33 vs 0.44, c/d, $p < 0.05$) preserved visual acuity 5 weeks following injection initially. At 12 weeks post-injection the protective activity for VA was not sustained in the V treated animals (OS vs OD: 0.26 vs 0.29, c/d, $p < 0.2$) whereas a sustained protective effect on VA decline was seen in group A (OS vs OD: 0.22 vs 0.47, c/d, $p < 0.01$). VA in hPRC line A treated eyes was better than those treated with V (0.47 vs 0.29, $p<0.05$). There was no difference between treated and untreated eyes in group B and D at both time-points. Statistical analysis of ONL measurement data demonstrated that hRPC line A led to significant preservation of the ONL compared with vehicle ($p<0.05$). Transplant survival was demonstrated in 2/10 Group A eyes. Transplanted cells were distributed throughout the neural retinal layers, including the ONL. Occasional differentiation into rhodopsin and opsin blue positive retinal phenotypes were observed within the surviving graft.
CONCLUSIONS: hRPCs were expanded successfully to P8. hRPC transplantation was well tolerated. No pathologies were noted at 12 weeks after cell treatment. Transplanting hRPCs to the subretinal space of RCS rats can be achieved with immunosuppression without obvious adverse reactions. hRPC line A showed promising results in arresting decline in VA over time in RCS rats , most likely achieved through host photoreceptor rescue rather than by cell replacement.

W-3168

LITHIUM INCREASES p63 LEVELS IN CULTURED HUMAN LIMBAL EPITHELIAL STEM CELLS.

Agate Noer, Erik O. Johnsen, Aboulghassem Shahdadfar, Charlotte L. Ness, Bjørn Nicolaissen, Morten C. Moe

Ophthalmology, Oslo University Hospital, OSLO, Norway

p63 (isoform ΔNp63α) is a putative stemness marker for limbal epithelial stem cells (LESCs), and is essential for the proliferative potential of stem cells in stratified epithelia. The niche for LESCs is the limbus, located between the clear cornea and bulbar conjunctiva. LESCs are responsible for and able to renew and repair the corneal epithelium throughout life. In a number of disorders and after trauma, limbal stem cell deficiency (LSCD) may occur, and this can be treated by isolation and cultivation of LESCs from a healthy eye followed by transplantation to the affected eye. It is known that the percentage of p63-bright cells affect the success rate of the transplantation. Thus, we wanted to reveal whether lithium, a known Wnt signaling inducing agent, may increase the expression of p63 in human LESC cultures.

Small limbal biopsies (1x1-2mm) were dissected from donor corneas and cultured in a standard medium at $37^{O}C$ and 5% CO_2 under different concentrations of lithium ($LiCl_2$). The cells were cultured for 3-6 weeks and medium was changed every 2-3 days.

The epithelial cells migrated from the limbal biopsy to form an epithelial sheet. Lithium enhanced the expression of p63 compared to controls, and this was confirmed by qRTPCR. Further studies mapping epigenetic alterations of the lithium treatment are currently conducted.Lithium induced upregulation of p63 in LESC could be clinically important by increasing the long term success of limbal stem cell therapy.

W-3171

DIFFERENTIATION POTENTIAL OF HUMAN LIMBAL FIBROBLASTS AND BONE MARROW MESENCHYMAL STEM CELLS INTO CORNEAL EPITHELIUM

Kishore Reddy Katikireddy, Reza Dana, Ula Jurkunas
Ophthalmology, Schepens Eye Research Institute, Mass. Eye and Ear, Boston, MA, United States

Purpose: Human limbal fibroblasts (LFs) and bone marrow mesenchymal stem cells (BM MSCs) are known for its multipotency. Specifically, a side population of stage-specific embryonic antigen-4 (SSEA4)-positive LFs has been shown to differentiate into a variety of cell types. The present study compared the stem cell characteristics of SSEA4+ and SSEA4- LFs to those of BM MSCs, and determined their potential to differentiate into the corneal epithelial phenotype.

Methods: Human cadaveric limbal tissue (n=6) was treated with dispase to remove the epithelium and endothelium. Single cell suspensions were obtained by digesting the stroma with 0.025% trypsin. Stem cell enrichment was performed by exposure of BM MSCs and LF cells in KnockOut ESC/iPSC medium for 12-15 days. LF and BM MSCs were sorted for SSEA4+ and SSEA4- cells by Magnetic-Activated Cell Sorting. Cell doubling time (CDT), stem cell (SC) marker analysis, and colony forming efficacy (CFE) were performed on sorted LF and BM MSCs. Epithelial phenotype was achieved using induction and differentiation media. Differentiated cells were characterized for corneal cytokeratins (CKs).

Results: After separation, enrichment SSEA4+ LFs 97.4 ± 0.6% and BM MSCs 93.5 ± 0.7% were achieved. The CDT of SSEA4+ LFs was 102 ± 1 hr and SSEA4- LFs was 58.2 ± 1.5 hrs ($p<0.02$). CDT of SSEA4+ BM MSCs was 105 ± 1 hr. and SSEA4- BM MSC was 56.3 ± 2 hrs ($p<0.02$). LF and BM MSC subgroups were negative for pan-cytokeratin. After enrichment, SSEA4+ cells showed the ability to form cell aggregates, while SSEA4- cells were mostly adherent to the culture plates. The transcript levels of SC markers OCT4, SOX2, Nanog and Rexo1 were higher in SSEA4+ than SSEA4- of LF and BM MSCs ($p<0.05$). Upon induction and differentiation, both SSEA4+ LFs and BM MSCs exhibited an epithelial morphology and positivity for CK3, CK12, and CK8, with high CFE ($p<0.001$), whereas the SSEA4- cells exhibited a fibroblast morphology and were negative for corneal epithelial markers.

Conclusion: Although both LFs and BM MSCs express stem cell markers and have some transdifferentiation potential, only SSEA4+ LFs clearly demonstrate the ability to differentiate into corneal epithelial cell phenotype. These findings establish a potential alternative source of corneal epithelial cells that can be harvested for ocular surface reconstruction.

W-3172

PARTIAL LIMBAL STEM CELL DEFICIENCY (LSCD) - DO WE NEED TO DISTURB THE FELLOW EYE?

Virender S. Sangwan[1], Jayesh A. Vazirani[1], Sayan Basu[1], Indumathi Mariappan[2]

[A]Cornea amd Uveitis Service, [B]Srikant and Sudhakar Stem Cell Laboatory, [1]L V Prasad Eye Institute, Hyderabad, India

Introduction:
Limbal stem cell deficiency(LSCD) clinically manifests with recurrent or persistent epithelial defects, vascularization, pannus formation, conjunctivalization of the corneal surface and loss of normal corneal clarity. Cell based therapy for restoration of the ocular surface in limbal stem cell deficiency has been utilized with good results at various centers across the world.
Purpose:
To report the outcomes of autologous cultivated limbal epithelial transplantation(CLET) utilizing the healthy part of the affected eye or the fellow eye as a source of limbal stem cells in patients with partial LSCD
Methods:
This retrospective study included 70 eyes of 70 patients with unilateral partial LSCD who underwent autologus CLET between 2001 and 2011. The limbal biopsy was taken either from the healthy part of the limbus of the same eye or from the healthy fellow eye. Cells were cultivated using a xeno free explant culture technique. Primary outcome measures were visual acuity and graft survival rate.
Results:
The mean follow up was 17.5 ± 7 months. The mean age of patients was 24 ± 12.5 years. Male:female ratio was 4:1. In 36 eyes the limbal biopsy was taken from the contralateral eye and in the remaining 34 eyes from the ipsilateral eye. The mean pre-operative visual acuity was 1.1 ± 0.6 logMAR, which improved to 0.8 ± 0.6 logMAR(p=0.04). The one year graft survival was 85 ± 7 percent in the contralateral group and 71 ± 8 percent in the ipsilateral group(p=0.74). Failure of limbal transplantation was seen in 9 eyes in the contralateral group and 10 eyes in the ipsilateral group.
Conclusions:
Ocular surface restoration in partial LSCD is possible with cell-based therapy. Outcomes are similar irrespective of whether the limbal biopsy is taken from the healthy part of the ipsilateral eye or the contralateral eye.

Neural Cells

W-3181

REDOX REGULATION OF NEURAL STEM AND PROGENITOR CELLS IN A MOUSE MODEL OF MATERNAL INFLAMMATORY RESPONSE CONTRIBUTES TO MILD BRAIN OVERGROWTH AND AUTISM-RELATED BEHAVIORS.

Janel Le Belle[1], Jantzen Sperry[1], Amy Ngo[1], Harley Kornblum[2]

[A]Psychiatry, [B]Psychiatry and Biobehavioral Sciences, Pharmacology and Pediatrics, [1]UCLA, Los Angeles, CA, United States

Autism Spectrum Disorders (ASD) is a heterogeneous set of developmental disorders characterized by deficits in social interaction and communication. Although ASD is highly heritable, the identification of its causes has been hindered by the heterogeneity of the syndrome. However, one of the more common endophenotypes in autism is brain overgrowth. Although there are known genetic causes of extreme brain overgrowth, a less severe but more pervasive brain overgrowth has been recently identified in retrospective studies using pediatric head circumference and MRI data. No mechanisms for this mild brain overgrowth or the specific behavioral consequences of the overgrowth are currently known. Despite the high heritability of ASD, there are also clear environmental components to autism pathogenesis and one of these known environmental risk factors is maternal inflammation, which may be caused by infection or other factors that induce an immunological response. Our study investigates neuroanatomical, cellular substrates, and the potential signaling mechanisms of brain overgrowth that may be associated with autism using a mouse model of Maternal Inflammatory Response (MIR). It is our hypothesis that mild MIR ex-

posure in early gestation can cause brain overgrowth via mechanisms dependent upon the NADPH oxidase (NOX) enzyme system and activation of the PI3K/AKT signaling pathway. Activation of this pathway is known to enhance neural stem and progenitor cell proliferation and neurogenesis. To test this hypothesis we developed an experimental model of low MIR that results in mild brain overgrowth and increased cortical thickness. We found the MIR-exposed offspring display abnormal autism-like behaviors which correlate with the amount of brain overgrowth. We show that this brain overgrowth is caused at least in part from an increase in stem and progenitor cell proliferation which we observed in the SVZ in vivo and in clonal assays in vitro. Even though our model of mild MIR exposure is based on a single injection of very low dose (8 ug/kg) lipopolysaccharide at an early gestational age (E9), we observe an increase in Iba1+ microglia throughout the brain post-natally. This suggests that there is a sustained immune activation in the brains of MIR-exposed offspring long after the initial exposure to MIR. Finally, we tested the hypothesis that the MIR-induced phenotype is dependent upon the production of reactive oxygen species generated by NOX and the subsequent activation of PI3K signaling by showing that brain overgrowth can be prevented by NOX inhibition.

W-3182

AUDITORY HAIR CELL FATE DETERMINATION BY COOPERATIVE ACTIVITY OF PAX2 AND SOX2

Judith S. Kempfle[1], Albert SB Edge[2]
[1]Otolaryngology, MEEI Harvard Medical School, Uni Tuebingen, Tuebingen, Boston, Germany, [2]Otolaryngology, MEEI, Harvard Medical School, Boston, MA, United States

The sound-sensing hair cells of the inner ear lack mechanisms for spontaneous regeneration. The cochlea harbors cells with stem cell properties, but even after damage they do not appear to be recruited for hair cell replacement. When cultured in vitro, inner ear stem cells proliferate and differentiate into hair cells, neurons and supporting cells. Two transcription factors, Pax2 and Sox2, play an important role in the differentiation of inner ear stem cells. Our data suggest synergistic effects of Pax2 and Sox2 on expression of transcription factor Atoh1, which is sufficient and essential for the formation of a hair cell from a sensory progenitor cell and is upregulated at the time that hair cells differentiate. Only mouse inner ear stem cells that co-expressed Pax2 and Sox2 in vitro differentiated into hair cells. Overexpression of Sox2 or Pax2 alone increased Atoh1 expression and the number of hair cells in vitro, but co-transfection of both factors led to robust up-regulation of Atoh1 and increased numbers of hair cells from inner ear stem cells. Our biochemical data show that both transcription factors are needed simultaneously in the same cell, where they co-bind to the regulatory region of Atoh1 to activate Atoh1 expression.
We show for the first time that Pax2 and Sox2 act together to activate Atoh1 expression and that both factors have a role in cell fate specification. Our data provide a mechanism that could form the basis for future approaches to regeneration of hair cells.

W-3183

INNER EAR STEM CELLS FOR TISSUE REGENERATION OF THE AUDITORY ORGAN

Marta Roccio, Stefan Hahnewald, Pascal Senn
Department of Clinical Research, University of Bern, Bern, Switzerland

Hearing impairment is the most frequent human sensory deficit and is mainly caused by the irreversible loss of neurosensory cells in the cochlea.
Previous studies have shown the presence of stem/progenitor cells within the mammalian auditory epithelium, vestibular organs and spiral ganglia, however the regenerative capacity of these organs is extremely poor, in contrast to birds and amphibians.
Stem/progenitor cells can be isolated through a sphere-forming assay in vitro or through prospective isolation using FACS and can give rise in vitro to the differentiated cell types of the tissue of origin. We are currently investigating a number of approaches to efficiently and reliably isolate and expand these cells from rodent as well as human adult and fetal tissues. Besides opening new venues in terms of tissue regeneration and repair, stem cell culture in vitro

provides a useful tool for drug screening to analyze hair cell regeneration and discover novel neurotrophic factors to increase auditory neurons survival.
Even if an exclusive stem-cell based hearing loss therapy may not be feasible in the future, stem cell technology can be used to improve current cochlear implant (CI) technology and therefore translate into a clinical benefit of hearing impaired individuals. We are developing a specifically modified CI electrode array in order to release neurotrophic and chemoattracting factors for induction of neuronal sprouting, guided migration and stable locking of the nerve endings on the electrode surface.
We show here how stem/progenitor cells isolated specifically from the spiral ganglia of early postnatal animals are in vitro expanded and further differentiated into auditory neurons and used for in vitro bioassays. Maturation is tested using marker expression and functionality through electrophysiological measurements on Multi Electrode Arrays (MEAs).
Our approach should lead to a deeper understanding of the regenerative potential within the human inner ear and to novel strategies for optimizing CI prostheses, leading to a gapless interface between the electrode and the neurons.

W-3184

MOTOR NEURONS GENERATED FROM ALS PATIENT-SPECIFIC IPS CELLS RECAPITULATE KEY ASPECTS OF DISEASE

Evangelos Kiskinis[1], Jackson Sandoe[1], Gabriella Boulting[1], Brian J. Wainger[2], Luis Williams[1], Clifford J. Woolf[2], Kevin Eggan[1]
[1]Stem Cell and Regenerative Biology, Harvard University, Boston, MA, United States, [2]FM Kirby Neurobiology Center, Boston Children's Hospital and Department of Neurobiology, Harvard Medical School, Boston, MA, United States

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurological condition characterized by selective death of motor neurons. Discovery of treatments for ALS and the understanding of mechanisms that cause it have been hampered by an inability to culture motor neurons isolated from patients and by the complex genetic nature of most cases. Patient-specific induced pluripotent stem cells (iPSCs), generated by epigenetic reprogramming of somatic cells, provide an invaluable tool for in vitro disease modeling, and offer a unique opportunity to study how genetic variants that cause neurological diseases modify the human, neural-types sensitive to degeneration. However, the extent to which iPSC-derived neurons are useful for studying adult onset conditions such as ALS, and whether any phenotypes observed in culture are due to disease-causing variants rather than idiosyncratic variation between iPSC-lines, remain significant questions. Here, we show that motor neurons produced from patient-derived iPSCs harboring the ALS-causing SOD1+/A4V mutation exhibited several phenotypes observed in the SOD1 mouse model and ALS patient histology. In particular, ALS-motor neurons decrease in numbers over long-term culture, with the largest motor neurons being selectively lost. Although ALS-derived motor neurons do not form large SOD1 aggregates, a typical pathological phenotype in post mortem patients, they exhibit signs of ER stress and mitochondrial impairment as well as altered electrophysiological properties. Importantly, these findings were absent in other neuronal types and were rescued following correction of the disease-causing genetic variant by homologous recombination, effectively demonstrating that these were direct effects of the ALS-causing mutation. Our results provide an insight to the functional defects that physiological levels of mutant SOD1 lead to, in patient motor neurons and add further credence to the notion that the promotion of protein-refolding is a therapeutic strategy worthy of consideration in SOD1-related ALS. More broadly our study demonstrates that iPSC technology can be used to probe an adult-onset neurological disease such as ALS.

W-3185

PATIENT-SPECIFIC IPS-DERIVED NEURAL MODEL FOR DD-EDS

Fengming Yue[1], Tomoki Kosho[2], Sakiko Shirasawa[3], Tadayuki Yokoyama[3], Daihachiro Tomotsune[1], Katsunori Sasaki[1]

[A]Dept of Histology and Embryology, [B]Department of Medical Genetics, [1]Medical School of Shinshu University, Matsumoto, Japan, [2]Bourbon Corporation, Kashiwazaki, Japan

Ehlers-Danlos
syndrome (EDS) is a heterogeneous connective tissue disorder involving skin and joint laxity and tissue fragility. Our colleague, Kosho established a new type of EDS, characterized progressive connective tissue fragility (skin hyperextensibility, joint hypermobility, deformity, subcutaneous hematoma), congenital abnormalities (craniofacial feature, delayed motor development, congenital multiple joint contractures), and CHST14 (CSs) mutation, deficiency of D4ST1(DSs), impairment of decorin-mediated assembly of collagen fibrils. It was named D4ST1-deficient EDS (DD-EDS, Kosho type). In this project, we establish patient-specific iPS-derived neural model for DD-EDS to demonstrate pathophysiological features in those mutant neurons. The undifferentiated status and pluripotency of established DD-EDS iPS cells were determined with Oct3/4, Nanog, SSEA-3 and SSEA-4 and teratoma formation. Cellular morphology was found different between DD-EDS-iPS and normal iPS cells, such as small size of cells, obscure cellular boundary, and many vacuoles in cytoplasm. In comparison to normal iPS cells, deficiency for CHST14 resulted in decreased neural progenitor development and diminished mature neurons. CSs and DSs have important roles in neural precursor proliferation, neuronal differentiation, migration and neuroprotection. This is evidenced by decreased DD-EDS iPS-derived neurogenesis, and consistent with clinical symptom, delayed motor development. Patient iPS cell-derived DD-EDS model will be helpful to reveal mechanism of rare heterogeneous disease and discover effective therapeutic drugs.

W-3186

EVALUATION OF HUMAN EMBRYONIC STEM CELL (HESC)/HUMAN INDUCED PLURIPOTENT STEM CELL (HIPSC)-DERIVED ASTROCYTES AS A MODEL SYSTEM TO STUDY APOLIPOPROTEIN E SECRETION

William L. Blake[1], Lorraine F. Lanyon[1], Claire M. Steppan[1], Sandra J. Engle[1], Kelly Bales[2], Gayathri Ramaswamy[2]
[1]Pfizer/PDM-NCE, Groton, CT, United States, [2]Pfizer/RU-Neuroscience, Cambridge, MA, United States

.
Apolipoprotein E (apoE) is an important lipid transport protein in plasma and brain. In the central nervous system (CNS), apoE is primarily produced by astrocytes and secreted as a high density lipoprotein-like particle. Human apoE has three isoforms: apoE2 (cys112, cys158), apoE3 (cys112, arg158), and apoE4 (arg112, arg158). ApoE is strongly associated with risk for developing Alzheimer's disease (AD) in a genotype dependent manner (apoE4>apoE3>apoE2) . ApoE has been found to play an important role in the removal of β-amyloid $_{1\text{-}42}$ from the brain, a key protein fragment responsible in part for the neuropathology of AD. Increased apoE production in animal models of AD has been associated with a reduction in β-amyloid $_{1\text{-}42}$. Human astrocytoma (CCF-STTG1) cells have been widely used as a model system to study apoE secretion in the CNS. Using the potent LXRα agonist T0901317 as a tool compound, we sought to evaluate the use of hESC and hiPSC derived astrocytes as a model system to study apoE secretion.
We derived astrocytes from both hESCs and hiPSCs, and compared them to primary human astrocytes and the astrocytoma cell line. The hESC/hiPSC-differentiation protocol entailed aggregate formation and subsequent plating of aggregates in the presence of small molecules to establish neural rosettes, treatment of resulting neuroprogenitors with epidermal growth factor (EGF) and ciliary neurotophic factor (CNTF) to obtain glial progenitors, and final differentiation to mature astrocytes by CNTF treatment.

The resulting hESC or hiPSC-derived cells expressed glial fibrillary acid protein (GFAP; 99% of cells), stained positive for astrocyte markers (s100β, glutamate transporters, excitatory amino acid Transporters 1 and 2), and secreted apoE. When treated with T0901317, astrocytoma cells showed a 17-fold increase (over vehicle) in apoE secretion at maximal dose (10μM). Similar treatment with T0901317 increased apoE secretion by 2.3-fold in primary human astrocytes . In stem cell derived astrocytes, the increase was more moderate (hESC: LXR -1.7-fold; hiPSC: LXR-1.4-fold).
Overall, the four astrocyte lines tested showed an increase in apoE secretion when treated with T0901317. However, the amount of apoE secreted differed among the various astrocyte lines. Our data suggest that hPSCs may be potentially developed into a model system to study apoE secretion.

W-3187

PSYCHOSINE ANTAGONIZES CRITICAL SURVIVAL AND PROLIFERATION PATHWAYS IN OLIGODENDROCYTE PROGENITOR CELLS: THERAPEUTIC IMPLICATIONS FOR KRABBE DISEASE

Christopher J. Folts, Nicole Scott, Mark Noble
Dept. of Biomedical Genetics, University of Rochester School of Medicine & Dentistry, Rochester, NY, United States

Lysosomal storage disorders (LSDs) represent some of the most devastating of genetic diseases. In one such LSD, Krabbe Disease, infants who present with the more prevalent early onset form of the disease typically do not survive beyond two years of age and have limited treatment options, most of which are palliative. The severe neurodegeneration and demyelination observed in patients has been attributed to the accumulation of the toxic sphingolipid psychosine, and both the accumulation of psychosine and myelin damage are observed in twitcher mice, a naturally occurring mouse model of the disease. Little attention has been paid, however, to the biology of stem and progenitor cells in these diseases. We found significant reductions in the pool of actively dividing oligodendrocyte progenitor cells (O-2A/OPCs)--cells needed for the generation of myelinating oligodendrocytes during CNS development and for tissue repair under conditions of myelin damage--in major white matter tracts of twitcher mice, even before symptoms are apparent. We also observed in primary O-2A/OPCs that pathophysiological exposure to psychosine in vitro is sufficient to cause cell death, as well as suppression of self-renewing division and differentiation at sublethal concentrations, via the antagonism of critical pro-survival and pro-mitotic signaling pathways. O-2A/OPCs are more sensitive to psychosine than oligodendrocytes, and appear to be the most sensitive cell type thus far identified. To develop possible new therapeutic interventions for Krabbe disease, we have undertaken a high-throughput, high-content screen of FDA-approved compounds and other compounds in primary O-2A/OPC progenitor cells, analyzing multiple parameters through use an adherent cell cytometer (Celigo™) enabling non-invasive, single-cell, FACS-type fluorometric analysis on adherent cells. We have identified 25 candidate protective agents through this multi-parameter screening effort. Although structurally- and functionally-diverse, these compounds share the ability to protect multiple essential cellular processes antagonized by psychosine. In vivo analysis of these compounds is ongoing. In sum, the O-2A/OPCs that are needed for CNS myelination and tissue repair are exquisitely sensitive to psychosine toxicities, both in vitro and in vivo. Using these cells as our starting point, we hope to expand our understanding of the cellular and molecular mechanisms of psychosine toxicity so as to yield novel therapies for the treatment of Krabbe Disease.

W-3188

DEVELOPMENT AND TRANSCRIPTOME PROFILING OF A NEURONAL MODEL OF LESCH-NYHAN DISEASE

Liam Crapper, Carolina Gigek, Gilles Maussion, Carl Ernst
McGill Group for Suicide Studies, Douglas Research Hospital, Montreal, QC, Canada

Lesch-Nyhan disease (LND) is a rare genetic disorder caused by the disruption of the gene HRPT1. LND has a variety of metabolic and neurological symptoms including crystals in the urine, gout, dystonia, intellectual disability, and chronic self-injury. While the high penetrance of the self-injury phenotype in LND is unique, similar challenging behaviors can be observed in many developmental disorders. The causes of neurological symptoms in LND remain

unknown and to date no treatments have been effective. Human post-mortem studies and animal models of LND show impaired dopaminergic functioning. These studies provide the strongest link between HPRT1 dysfunction and the neurological symptoms of LND; however, dopaminergic impairment alone is not sufficient to cause self-injury. We have developed a novel model of LND using a shRNA knockdown of HPRT in immortalized fetal neural progenitor cells from the ventral midbrain. We have differentiated the progenitors into mature neurons and examined global alterations in gene expression using RNAseq. This study is the first to use high-throughput analysis in a neuronal model of LND, and will provide information vital to the understanding of LND pathogenesis and show pathways which may be involved in the challenging behaviors of other developmental disorders.

W-3191

THE DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS CULTIVATED ON EPIDERMAL GROWTH FACTOR-LOADED SCAFFOLDS TOWARD FUNCTIONAL NEURONS UNDER NEURONAL INDUTIVED MEDIUM

Thayane Crestani[1], Kerlin Quintiliano[1], Virginia Etges Helfer[2], Davi Silveira dos Santos[3], Eduardo A. Balbueno[2], Estela Mitie Cruvinel[4], Antonio Carlos Cassola[4], Geraldo Jotz[5], Diogo André Pilger[2], **Patricia Pranke**[6]

[1]Hematology and Stem Cell Laboratory, Faculty of Pharmacy; Post-graduate Program in Neuroscience - Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [2]Hematology and Stem Cell Laboratory, Faculty of Pharmacy; Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [3]Hematology and Stem Cell Laboratory, Faculty of Pharmacy; Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [4]Biosciences Institute, Federal University of São Paulo (USP), São Paulo, Brazil, [5]Post-graduate Program in Neuroscience; Post-graduate Program in Physiology - Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [6]Hematology and Stem Cell Laboratory, Faculty of Pharmacy; Post-graduate Program in Physiology - Federal University of Rio Grande do Sul; Stem Cell Research Institute, Porto Alegre, Brazil

A strategy to optimize tissue regeneration is the use of nanofiber matrices containing growth factors (GFs). This strategy is to ensure the correct distribution of stem cells, enhancing their proliferative and differentiation capacity at the injury site, thus preventing the cells from migrating to other locations. The bioavailability of these factors can be obtained by the incorporation of GFs into nanofibers by the electrospinning technique. This aim of this work has been to produce aligned nanofiber matrices with incorporated epidermal GF (EGF) to evaluate their influence on the differentiation of mesenchymal stem cells into neural precursors. Aligned scaffolds with and without EGF were produced by electrospinning, using emulsion consisting of poly(lactic-co-glycolic acid) (PLGA) 15% (w/w), Span-80 0.2%, 1,1,1,3,3,3-Hexafluoro-2-propanol with an aqueous phase containing PBS, albumin (BSA) and 1mg/mL of EGF. The matrices were evaluated for morphology fiber diameter and degradability. Stem cells from human exfoliated deciduous teeth (SHEDs) were cultivated on the scaffolds and were evaluated for the following biological tests: (1) cell adhesion after 6 hr incubation by using DAPI staining, (2) cell viability analyzes after 6, 24, 48 and 72 hours cultivation by MTT assay, (3) cytotoxicity assay on days 4, 7, 14 and 21, through the dosage of the enzyme lactate dehydrogenase (LDH) and (4) neuronal differentiation by phase contrast microscopy, immunofluorescence and electrophysiology. The control group was the cells cultured on wells directly on the culture plates. PLGA/BSA and PLGA/BSA/EGF scaffolds showed aligned fibers, absence of beads, with an average diameter of 537±73nm and 581±79 nm, respectively. There is no difference between the morphology and diameter of both scaffolds ($p<0.05$). The adhesion assay showed that the cells adhere more on PLGA/BSA/EGF than PLGA/BSA scaffolds (p 0.05). Using confocal microscopy, it is possible to observe that SHEDs adhere onto scaffolds, following the same orientation of aligned fibers. The amount of metabolically active cells cultivated, using MTT assay, on both scaffold groups was similar ($p<0.05$). On all days of analyzes both scaffolds were similar to the control group and different from the Triton group, which represents the maximum release of LDH ($p<0.001$). Therefore, this data confirms that the scaffolds are atoxic for the SHEDs. Concerning neuronal differentiation, samples cultivated on both scaffolds and on the control group showed cells with neural phenotype, labeling positively for nestin and GFAP markers. Analyzes are being made to quantify the number of cells positive for each marker in relation to the total number of cells to evaluate the real influence of the alignment of the fibers and EGF in neurogenesis. An increased number of cells

cultivated on PLGA/BSA/EGF, in comparison with other groups, exhibited the capacity to produce sodium current consistent with functional neuronal cells when exposed to neuronal inductive media. Both scaffolds appeared to be good supports for cell adhesion and viability with low cytotoxicity. Scaffolds produced with the incorporation of the EGF showed an improvement in cell adhesion and presence of sodium channels. Therefore, the suggestion is that these matrices can be a possible option for use as biomaterials in neural tissue engineering because they provide stimulus for the cells supporting cellular connectivity.

W-3192

ASSOCIATION BETWEEN MESENCHYMAL STEM CELLS, FROM HUMAN EXFOLIATED DECIDUOUS TEETH PULP, AND ELECTROSPUN SCAFFOLDS AS A STRATEGY FOR NERVOUS SYSTEM INJURIES

Kerlin Quintiliano[1], Thayane Crestani[1], Davi Silveira[2], Virginia E. Helfer[2], Annelise Rosa[3], Eduardo Balbueno[2], Daniela Steffens[3], Geraldo P. Jotz[4], Diogo A. Pilger[2], **Patricia Pranke**[5]

[1]Hematology and Stem Cell Laboratory, Faculty of Pharmacy; Post-graduate Program in Neuroscience - Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [2]Hematology and Stem Cell Laboratory, Faculty of Pharmacy - Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [3]Hematology and Stem Cell Laboratory, Faculty of Pharmacy; Post-graduate Program in Material Science - Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [4]Post-graduate Program in Neuroscience; Department of Morphological Sciences - Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [5]Hematology and Stem Cell Laboratory, Faculty of Pharmacy; Post-graduate Program in Material Science - Federal University of Rio Grande do Sul; Stem Cell Research Institute, Porto Alegre, Brazil

Scaffolds produced by the electrospinning technique can act as supports for proliferation and differentiation of mesenchymal stem cells (MSCs), providing an alternative for application in regenerative medicine. The scaffold process of nervous tissue regeneration can be improved through the presence of neurotrophic factors such as NGF (Nerve Growth Factor), which plays a central role in the development, maintenance and survival of neurons. This study has aimed to develop scaffolds with aligned and random nanofibers with and without incorporated NGF and to evaluate biological and physicochemical parameters. MSCs were extracted from human deciduous teeth pulp. The following groups were developed and characterized, scaffold with: random nanofibers control (RC), random nanofibers with NGF (RN), aligned nanofibers control (AC) and aligned nanofibers with NGF (AN). The physicochemical analyzes performed were nanofiber morphology and diameter. The biological parameters assessed on the scaffolds were cellular adhesion and proliferation, cytotoxicity, as well as neural differentiation (gene expression for enolase, β-III tubulin and nestin). In all the groups, nanofibers with homogeneous morphology without beads were observed. The nanofiber average diameter was 610±174 nm, 585±109 nm, 537±73nm and 543±128 nm for RC, RN, AC and AN scaffolds, respectively ($P > 0.05$). The adhesion test showed no significant difference between the four groups of scaffolds and the control group (cells cultured directly on well culture plates). This indicates a satisfactory interaction between the cells and the scaffolds, as they showed a similar adhesion pattern to the control. The LDH quantification demonstrated that all groups of scaffolds were similar to the negative control ($P>0.05$), suggesting that they are nontoxic for cells for the 21 days evaluated. All scaffold groups showed similar proliferation rates to the control group at both periods assessed (4 and 7 days). This shows that cell proliferation is sustained when MSCs are cultured on these structures. For neural differentiation analysis, MSCs were cultured on scaffolds or well culture plates for 21 days with neural induction media or with conventional media for MSC culture (control). Gene expression of NSE (Neuron Specific Enolase), β-III tubulin and nestin presented a similar pattern in the MSCs cultivated on the AC scaffold group, cultivated without neural induction media, when compared to the gene expression in the MSCs cultivated with neural induction media seeded directly on well culture plates. This indicates that aligned nanofiber scaffolds could possibly stimulate MSCs for neural differentiation with no other stimuli except the alignment itself. Nestin and NSE expression were up-regulated while β-III tubulin was down-regulated in all scaffold groups not subjected to neural induction. When MSCs were cultured on scaffolds with neural induction media, nestin upregulation was only evident in the AN scaffolds showing a 2.6-fold increase, possibly influenced by NGF presence. MSCs on AC scaffolds treated with neural induction media up-regulated NSE and down-regulated β-III tubulin and nestin expression. This could indicate cellular mechanisms underlying mature neuronal differenti-

ation. This data suggests that the association between MSCs and scaffolds are appropriate candidates for further evaluation as neural tissue engineering strategies using animal models representative of nervous system injuries.

W-3193

SOX6 REGULATED BY MACROPHAGE MIGRATION INHIBITORY FACTOR PROMTES CELL SURVIVAL AND MAINTENANCE OF MOUSE NEURAL STEM/PROGENITOR CELLS

Shigeki Ohta[1], Aya Misawa[1], Hideyuki Okano[2], Yutaka Kawakami[1], Masahiro Toda[3]
[A]Div of Cellular Signaling, Inst for Adv Med Res, [B]Physiology, [C]Neurosurgery, [1]Keio Univ School of Medicine, Tokyo, Japan

Macrophage migration inhibitory factor (MIF) was identified as a factor that can support the proliferation and/or survival of murine neural stem/progenitor cells (NSPCs) in our previous study. We newly discovered that MIF increases the expression of Sox6, but not Sox1 or Sox2 gene in NSPCs *in vitro*. During neural development, Sox6 was expressed in the ventricular zone of ganglionic eminence (GE) of mouse brains at embryonic day 14.5 (E14.5), cultured NSPCs from E14.5 GE, and NSPCs and neural progenitor cells in the sub-ventricular zone (SVZ) around the lateral ventricle (LV) of the adult mouse forebrain. Retrovirally-expressed Sox6 in NSPCs increased cell viability and the number of primary and secondary neurospheres, thereby supporting cell survival and/or self-renewal ability. The differentiation potential of NSPCs was inhibited by retroviral Sox6 overexpression in NSPCs. Sox6 increased Hes1 and Bcl-2 expression and the phosphorylation of Akt in NSPCs. MIF was shown to activate Stat3 in NSPCs in our previous study. Constitutively-activated Stat3 up-regulated Sox6 gene expression and ChIP analysis showed that MIF increased Stat3 binding to the Sox6 promoter in NSPCs, indicating that Stat3 is a downstream molecule regulated by MIF which promotes Sox6 gene expression. The ability of MIF, which can increase the number of primary and secondary neurospheres was inhibited by Sox6 gene silencing, suggesting that Sox6 may play a key role as a downstream molecule of MIF to support NSPC stemness.

W-3194

MODULATING THE KYNURENINE PATHWAY TO PROMOTE STEM CELL REPAIR IN MULTIPLE SCLEROSIS.

Chai K. Lim[1], **Simon Jones**[2], Juliana Croitoru-Lamoury[2], Gilles J. Guillemin[1], Bruce J. Brew[3]
[1]Australian School of Advance Medicine, Macquarie University, Sydney, Australia, [2]St Vincent's Centre for Applied Medical Research, The University of New South Wales, Sydney, Australia, [3]Department of Neurology, St Vincent's Hospital, Sydney, Australia

Background: The current therapeutic strategies for multiple sclerosis (MS), such as IFN-β, are of limited efficacy. There exist no treatments that can regenerate or repair damaged myelin and therefore reverse neurological disability in MS. The kynurenine pathway (KP) is a major metabolic pathway that catabolises tryptophan. Tryptophan metabolism is aberrant in a number of inflammatory conditions, including MS. The KP yields several neuroactive downstream metabolites; one of the most biologically significant is quinolinic acid (QUIN). Our group has previously shown QUIN to be neurotoxic and gliotoxic at concentrations of >150nM and >350nM respectively. We have also demonstrated that the KP is fully expressed and functional in both mouse and human mesenchymal stem cells and partially in neural stem cells (NSC). Furthermore the KP is activated by type I & II interferons.
Aim: To explore the potential of modulating the KP to optimise stem cell function therefore promoting remyelination of neurons. We hypothesise that chronic activation of the KP impairs the proliferation and differentiation of NSC.
Methods: Murine NSC were cultured as previously described in our lab and treated with 100 IU/ml IFN-β alone or in combination with 50 nM QUIN. Levels of tryptophan, kynurenine and QUIN were measured in mice with EAE, the widely accepted murine model of MS. Tryptophan and kynurenine were quantified using HPLC. This was expressed as kynurenine:tryptophan (K/T) ratio; a reliable measure of KP activation. Concentrations of QUIN were assessed using GCMS.

Results: Treatment of NSC with IFN-β significantly attenuated their proliferation. Interestingly the addition of QUIN, at a basal concentration of 50 nM, recovered NSC proliferation *in vitro.* Furthermore we found that mice with EAE had an activated KP, as reflected by an increased K/T ratio of 27.14 ± 6.67 in diseased mice compared to 16.6 ± 2.12 in control animals. We also observed that *in vivo* levels of QUIN increased almost two-fold with EAE progression from 129.64 nM ± 18.93 to 227.82 nM ± 54.06 (n=8).
Conclusions: We have shown that NSC are strongly influenced by endogenous KP metabolites and, in the right KP metabolic conditions, can proliferate appropriately. However, in mice with EAE the KP is significantly activated - as measured by an increased K/T ratio and elevated QUIN. Chronic activation of the KP generates neurotoxic levels of QUIN and reduces available tryptophan. This is likely to affect NSC proliferation, differentiation and subsequent remyelination. Results here provide a vital platform for investigating how manipulation of the KP in stem cells could be crucial for the repair of neurons in MS.

W-3195

ENHANCED REMYELINATING AND IMMUNOMODULATORY EFFECTS OF NEURAL STEM CELLS BY TRANSDUCTION OF NEUROTROPHIN 3

Jingxian Yang[1], Guang-Xian Zhang[2]
[1]Department of Pharmacology, Liaoning University of Traditional Chinese Medicine, Dalian, China, [2]Department of Neurology,, Thomas Jefferson University, Philadelphia, PA, United States

The therapeutic potential of neural stem cells (NSCs) on experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS), has been recently suggested; however, the clinical and pathological improvement is limited. To enhance their therapeutic effect, we in the present study transduced bone marrow derived NSCs (BM-NSCs) with Neurotrophin 3 (NT-3), a potent neurotrophic factor that possesses both neuroprotection and immunomodulation capacities, with a novel Tet-on system to control NT-3 expression. We provide evidence that this approach significantly reduced central nervous system (CNS) inflammation and neurological deficits of ongoing EAE compared to conventional NSC therapy, together with the following advantages: 1) Enhanced BM-NSC proliferation and differentiation of oligodendrocytes and neurons, while inhibited differentiation of astrocytes, thus promoting remyelination and neuronal repopulation, and reducing astrogliosis; 2) Enhanced the anti-inflammatory capacity of BM-NSCs, thus more effectively suppressed CNS inflammation and accelerated endogenous and exogenous remyelination; 3) The easy accessibility of BM-NSCs provides another advantage over brain-NSCs for MS therapy. 4) A Tet-on system can control NT-3 expression as desired. Thus, our study provides a novel approach to break the vicious inflammation-demyelination cycle, and pave the way to an easily accessible, inducible and highly effective therapy for CNS inflammatory demyelination.

W-3196

HUMAN PLURIPOTENT STEM CELL-DERIVED RADIAL GLIAL LIKE CELLS WITH STABLE REGIONAL IDENTITIES

Laura Ostermann[1], **Julia Ladewig**[1], Franz-Josef Müller[2], Philipp Koch[1], Oliver Brüstle[1]
[1]Reconstructive Neurobiology, Bonn, Germany, [2]Department of Psychiatry and Psychotherapy,, Centre for Integrative Psychiatry,, Kiel, Germany

Key challenges associated with the biomedical application of pluripotent stem cell (PSC)-derived neural cells are their controlled patterning towards distinct regional subtypes and the maintenance of an acquired regional phenotype across multiple passages of in vitro expansion. Previous studies have shown that rosette-type long-term self-renewing neuroepithelial stem cells (lt-NES cells) generated from human PSC undergo gradual posteriorization into an anterior hindbrain phenotype, which might be due to regionalizing effects of the growth factors employed for in vitro proliferation (Koch et al., PNAS 106:3225-30, 2009). During normal CNS development, early NES cells give rise to radial glia (RG) cells, which represent a major source of regionally determined neurons in the embryonic and fetal brain. Here we explored whether PSC-derived neural stem cells (NSC) can be coaxed into RG-like cells

and whether those maintain a stable regional phenotype. Using partially differentiating conditions we were able to establish a NSC population, which exibits features of multipotent neuro- and gliogenic RG cells. These RG-like NSC could be expanded for at least 25 passages and expressed classical NSC markers such as nestin and SOX2 as well as markers typically associated with RG including SOX9, CD44, AQP4 and HOP, while the lt-NES cell markers PLZF, DACH1, ZO-1 and MMRN1 were down-regulated. We next asked whether these cells retain their regional phenotype during in vitro proliferation. To that end we generated RG-like cells from anterior, hindbrain- and spinal cord-patterned rosette-type NSC. We found that RG-like cells with those regional identities continue to express transcription factors appropriate for their positional identity across multiple passages of in vitro expansion while maintaining their differentiate potential into neurons and glia. Thus, conversion into RG-like cells may provide a route for conserving the regional identity of pre-patterned early NSC.

W-3197

THE 9-O-ACETYL GD3 GANGLIOSIDE AS A SURFACE MARKER FOR NEURAL PROGENITOR CELLS

Marcelo F. Santiago, Fernanda Gubert, Camila Zaverucha-do-Valle, Michelle Furtado, Nicoli Mortari, Bruno Diaz Paredes, Rosalia Mendez-Otero
Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

In adult mammals, neurogenesis persists in two regions: the subventricular zone (SVZ) around the lateral ventricles and the subgranular zone in the hippocampus dentate gyrus. Neural stem cells can be isolated from these areas and are characterized as self-renewable multipotent cells capable of originating neurons, astrocytes and oligodendrocytes. In vitro, these cells can be identified using neurosphere assays, but in vivo their precise identity remains unclear because exclusive markers have not yet been found. The 9-O-acetyl GD3 ganglioside (CD60b) was described as an antigen associated with cellular migration and axonal extension during the development of the nervous system. In the adult, the ganglioside expression is down regulated in most of the brain but remains in a very few specific regions including the SVZ. In this work, we have used magnetic activated cell sorting (MACS) to isolate highly expressing 9-O-acetyl GD3 (CD60b+) cells from the SVZ and we compared the neurosphere formation in CD60b+ and CD60b- populations. We analyzed cells obtained from rats in three different ages: E16, P21 and P90 (adult). In E16, there was no significant difference in neurosphere diameter and in the number of formed neurospheres comparing CD60b+ and CD60b- populations. In P21 and P90 animals, however, the CD60b+ cells were able to form a higher number of neurospheres when compared to the CD60b- population. In P21, the mean neurosphere diameter was also higher in the CD60b+ population.
Furthermore, the phenotypic analysis of the adult SVZ shows that CD60b+ cells do not bind to the PNA (peanut agglutinin) antigen, an excluding marker for NSCs. On the other hand, a subpopulation of CD60b+ cells co-label with the early neuroepithelial intermediate filament Nestin and the neuroblast marker doublecortin.
Based on these results we show that the 9-O-acetyl GD3 ganglioside is expressed in a subset of neural stem cells/progenitor cells in the young/adult SVZ and we suggest that this antigen could be used as a surface marker for isolation/enrichment of adult NSCs.

W-3198

AN INDUCED PLURIPOTENT STEM CELL MODEL OF NEUROTOXICITY IN PROGRANULIN-DEFICIENT FRONTOTEMPORAL DEMENTIA

Amanda R. Mason[1], Sami Barmada[1], Lauren H. Martens[2], Xialian Xu[2], Aaron Daub[1], Robert V. Farese, Jr[2], Steven Finkbeiner[1]
[A]Gladstone Institute of Neurological Disease, [B]Gladstone Institute of Cardiovascular Disease, [1]Gladstone Institutes, San Francisco, CA, United States

Frontotemporal Dementia (FTD) is the most common dementia in patients younger than 60. FTD patients exhibit behavioral changes or language impairment and progress to death in 6-9 years. No effective treatments exist. Disease symptoms are caused by massive neuronal loss in the frontal and temporal lobes. Among the known ge-

netic causes of FTD, Progranulin (PGRN) haploinsufficiency is one of the most common. The goal of this study is to model neuronal death caused by PGRN haploinsufficiency using cortical neurons derived from FTD patient induced pluripotent stem cells (iPSCs). We present preliminary data that FTD patient fibroblasts can be reprogrammed to iPSCs and then differentiated to neuronal subtypes. Additionally, neurons from mice lacking PGRN exhibit time-dependent neurodegeneration in culture. Exogenous PGRN can rescue neurodegeneration and affects protein clearance pathways in cortical neurons, suggesting that protein homeostasis is critical for maintaining neuronal health in FTD. Future studies will investigate survival and proteostasis phenotypes in iPSC-derived neurons.

W-3201

PREDICTING THE SUCCESS OF POTENTIAL THERAPEUTICS FOR ALS USING PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

Kevin J. Kim, Pamela A. Schein, Lee L. Rubin
Dept. of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a fatal neurodegenerative disease affecting 20,000 to 30,000 people in the U.S., with an estimated 5000 new cases every year. Following degeneration of both upper motor neurons from the motor cortex and lower motor neurons from the brainstem and spinal cord, ALS patients undergo loss of motor function and eventually die from respiratory failure within 3 to 5 years from the onset of symptoms. While most ALS cases are sporadic, about 10% of the cases are familial, with mutations in the genes superoxide dismutase 1 (*SOD1*), TAR DNA-binding protein 43 (*TDP-43*), and *C9ORF72*. The only FDA-approved drug for ALS, Riluzole, has, at best, a modest effect, extending lifespan by 3 to 5 months. Therefore, discovering new and better therapeutics is quite important. Yet, despite some mechanistic insights obtained from studying some of the familial cases, past drug discovery attempts have not yielded successful therapies. The recent failures of Olesoxime and Dexpramipexole in Phase III clinical trials reveal the limitation of conventional approaches to finding truly effective ALS treatments. In part, this may be because many clinical candidates that show efficacy in the standard murine models of ALS have failed in the clinic, suggesting that this ALS model is not sufficiently predictive of the response of ALS patients. In addition, the heterogeneity of the disease may complicate the identification of individual patient response to specific agents. To better address these issues, we established a platform using disease relevant cells derived from induced pluripotent stem cells (iPSCs) of multiple patients to serve as the basis for testing potential therapeutics that may rescue the disease types found in patient subpopulations. IPSCs from control and ALS patients were differentiated into enriched populations (>50%) of motor neurons and cultured on mouse glial cells to provide an *in vitro* environment similar to that of the adult CNS. The co-culture was optimized for an automated 384-well assay and quantified using a high content imaging system. Testing of a selection of previous clinical drug candidates showed varying effectiveness of each compound across different lines of ALS motor neurons. In this study, we demonstrate that disease-relevant cells derived from an array of patient-specific iPSCs can be used to test the effectiveness of potential therapeutics towards different forms of ALS and may serve as a valuable and more predictive preclinical model.

W-3202

RAPID NEUROEPITHELIUM DERIVATION FROM HUMAN PLURIPOTENT STEM CELLS USING FULLY DEFINED, SMALL MOLECULE INHIBITOR INDEPENDENT, AND ADHERENT CULTURE CONDITIONS

Ethan Lippmann, Maria Estevez-Silva, Randolph Ashton
Biomedical Engineering, University of Wisconsin-Madison, Madison, WI, United States

The embryonic neuroepithelium gives rise to the entire central nervous system (CNS) *in vivo*, making it an important tissue for developmental studies and a prospective cell source for regenerative applications. Current protocols for deriving highly pure neuroepithelial cultures from human pluripotent stem cells (hPSCs) *in vitro* consist of either embryoid body-mediated neuralization followed by a selective, manual isolation step or adherent differentiation using small molecules to suppress mesendodermal fates and promote neuroepithelium formation upon passaging.

Herein, we demonstrate a protocol where hPSCs maintained under defined conditions can be differentiated in an adherent monolayer to yield neuroepithelium (>90% $Pax6^+$/N-$cadherin^+$) within 6 days using a medium consisting of only DMEM/F12, sodium bicarbonate, selenium, ascorbic acid, transferrin, and insulin. Using this method, H9 human embryonic stem cells (hESCs) previously cultured under defined conditions routinely reach purities of 99-100% $Pax6^+$ neuroepithelium regardless of whether they are differentiated on an undefined (Matrigel) or defined (recombinant vitronectin peptide) substrate. In contrast, hPSCs maintained on mouse embryonic fibroblasts (MEFs) exhibit much lower neuroepithelial differentiation efficiency using this method (1-40% $Pax6^+$), but transferring the hPSCs to feeder-independent conditions for two passages is sufficient to regain high efficiency. The hPSC-derived neuroepithelium defaults to anterior forebrain identity and can be rapidly patterned to caudal ($HoxB4^+$) and ventral ($Nkx6.1^+$) fates, indicating the neuroepithelium is responsive to lineage patterning cues. Overall, because this protocol is defined and requires no small molecule inhibitors or manual enrichment steps, it can be easily scaled and widely used to derive neuroepithelia from which large quantities of neural cells can be generated for basic and translation research.

W-3203

TRANSCRIPTOMES OF PROLIFERATING NEURAL STEM CELLS, DIFFERENTIATING PROGENITORS AND NEWBORN NEURONS IDENTIFY LONG NON-CODING RNAS AS NOVEL PLAYERS IN CORTICOGENESIS

Julieta Aprea Perez[1], Silvia Prenninger[1], Tanay Ghosh[2], Dimitra Alexopoulou[1], Elke Wessendorf[1], Andreas Dahl[1], Matthias Groszer[2], Michael Hiller[3], Federico Calegari[1]

[1]DFG–Research Center and Cluster of Excellence for Regenerative Therapies, Dresden, Germany, [2]Institut du Fer à Moulin, INSERM UMR-S 839, Université Pierre & Marie Curie, Paris, France, [3]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Transcriptome analysis of somatic stem cells and their progeny is fundamental to identify the molecular mechanisms regulating the transition from proliferation to differentiation. However, analysing transcriptomes of individual cell types in complex tissues remains a challenge. We generated a RFP/GFP double-reporter mouse line to isolate proliferating neural stem cells, differentiating progenitors and newborn neurons that coexist as intermingled cell populations in the developing cortex. Transcriptome sequencing of these three cell types revealed numerous uncharacterized protein-coding genes and several long non-coding (lnc)RNAs with highly specific and transient expression patterns. Most identified lncRNAs overlapped genes implicated in neurogenesis and shared with them a nearly identical expression pattern suggesting that lncRNAs control neural stem cell differentiation by regulating the expression of cell fate determinants. Finally, we investigated the function of one lncRNA during cortical development and found that it is involved in neurogenic commitment of neural progenitors as well as survival of newborn neurons. Our study provides the most comprehensive and quantitative transcriptome resource during the switch of neural stem from proliferation to differentiation to date and identifies crucial roles of lncRNAs during mammalian corticogenesis.

W-3204

EFFICIENT NON-VIRAL REPROGRAMMING OF MOUSE FIBROBLASTS DIRECTLY INTO NEURONS

Ellen Amalia Force, Joseph LoTurco
Physiology and Neurobiology, University of Connecticut, Storrs, CT, United States

Mouse embryonic fibroblasts (MEFs) can be directly reprogrammed into neurons with the introduction of three transcription factors, Ascl1, Brn2 and Myt1L, by virally-mediated transgenesis (Vierbuchen et al. 2010). This dramatic type of direct reprogramming, independent of passing through induced pluripotent stem cell stages, presents unique opportunities for exploring control of neuronal identity and the production of neurons from non-neural proliferative cells. In this study, our goal was to test whether induced neurons (iNs) could be produced by trans-

fection of plasmid DNA, bypassing the use of virus and genomic integration of transgenes. We used the liposomal transfection reagent FuGene 6 to transfect Tau-EGFP MEFs with plasmids encoding the three transcription factors, Ascl1, Brn2 and Myt1L. Each expression plasmid contained the ubiquitously active CAG promoter, the cDNA for each respective transcription factor, a polyadenylation signal, and two piggyBac transposon terminal repeats flanking the entire cassette. On their own, PB-pCAG-Ascl1, PB-pCAG-Brn2 and PB-pCAG-Myt1L can be used as episomal expression plasmids, or in combination with a plasmid expressing piggyBac transposase (pCAG-PBase) they can be used to non-virally deliver genomically integrated transgenes. Thus, MEFs were transfected with either BAM alone, or along with pCAG-PBase, to compare the effectiveness of genomically integrated plasmids to episomal plasmids. Five days post-transfection, cells were switched from a fibroblast media to a neural differentiation media. Cells were fixed 7, 10 and 13 days post-transfection, and assessed for the presence of neurons by GFP and β-III-tubulin (Tuj1) expression. Seven days post-transfection few Tuj1+ cells were observed in transfections without pCAG-PBase (~400 Tuj1+ cells/well). The morphologies of these cells at day 7 were generally quite simple, with minimal branching on few processes, but became increasingly more complex with longer and more branched processes by 13 days post transfection. Cells given the pCAG-PBase displayed the same progression of growth and complexity, but were present in approximately double the number as those without pCAG-PBase. At day 7, ~750 Tuj1+ cells/well were observed, and this increased to ~1500 cells by day 13. These results show that MEFs can be directly reprogrammed into neurons by transfection of plasmid DNA and may not require genomic integration of transgenes.

W-3205

INTERMITTENT ENVIRONMENTAL ENRICHMENT: A NOVEL STRATEGY FOR UNTANGLING THE VARIABLES REGULATING ADULT HIPPOCAMPAL NEUROGENESIS

Catherine-Alexandra Gregoire, David Bonenfant, Adalie Le Nguyen, Anne Aumont, Karl J.L. Fernandes
Universite de Montreal, Montreal, QC, Canada

The hippocampal dentate gyrus (DG) is a rare niche where neurogenesis is preserved throughout life and in which the neurons are implicated in the processes of learning and memory. Remarkably, adult neurogenesis is highly stimulated by environmental enrichment (EE), including variables such as running, environmental complexity, social interactions and stress, which exerts powerful effects on brain physiology. However, the relative contribution of individual EE variables to hippocampal neurogenesis remains a longstanding and poorly understood issue. To help untangle the effects of these variables, we developed a novel experimental paradigm based on intermittent exposure to EE. Adult male CD1 mice were alternated daily between two environments for 4 weeks; by comparing experimental groups in which only one of the two environments differed, individual EE variables could be effectively isolated. We found that intermittent voluntary exercise was sufficient to maximally increase the proliferation, neuroblast and post-mitotic neuron stages of neurogenesis, and to elicit changes in depolarization-associated c-fos expression within the dentate gyrus. Surprisingly, neither social enrichment nor chronically increased levels of stress-associated plasma corticosterone had a persistent impact on neurogenesis. Moreover, environmental complexity (inanimate objects, social enrichment and novelty) effectively buffered corticosterone increases, yet did not affect basal or running-induced neurogenesis. Mouse strain, handling and type of running apparatus were excluded as potential confounding variables. These findings help resolve the effects of distinct variables on adult neurogenesis, and this intermittent EE paradigm will serve as a useful tool for rationally designing more effective and targeted EE approaches.

W-3206

HNK-1 IDENTIFIES A SUBPOPULATION OF IMMATURE NEURONAL CELLS WITH PROLIFERATION CAPACITY IN THE ADULT MAMMALIAN CAROTID BODY

Veronica Sobrino, Ricardo Pardal
Fisiología Médica y Biofísica, Instituto de Biomedicina de Sevilla (IBiS). Hospital Universitario Virgen del Rocío/CSIC/ Universidad de Sevilla., Sevilla, Spain

The carotid body (CB) has recently been described as a neurogenic niche in the adult mammalian peripheral nervous system (PNS). This peripheral chemoreceptor organ is responsible for the detection and communication of hypoxemia to the central nervous system (CNS). Under maintained low O2 conditions, the CB contributes to physiological adaptation of the organism by increasing three-fold its size, mainly due to an increase in the number of chemoreceptor neuronal cells. This physiological neurogenesis has been partially identified as the result of proliferation and differentiation of a resident population of CB stem cells (CBSCs) within this niche. However, we cannot discard a parallel proliferation of neuronal cells, since different groups have described cell divisions in CB neuronal cells under hypoxic conditions.
By combining immunohistochemistry and flow cytometry techniques, we have found that the human natural killer-1 (HNK-1) glyco-epitope identifies, among other positive cells, a subpopulation of neuronal cells with proliferation capacity and expression of low levels of the differentiation gene, tyrosine hydroxylase (TH). In response to chronic hypoxia, these cells divide and complete differentiation into TH+ (HNK-1 negative) neuronal cells, to contribute to the physiological adaptation of the organ.
Proliferation and differentiation of these neuroblast-like immature neuronal cells is triggered by hypoxia. However, we have found that this activation process needs hypoxia-induced mature neuronal cell exocytotic activity. Blocking of exocytosis with the voltage-gated calcium channel blocker cadmium abrogates the process. Hence, we have demonstrated the existence of a subpopulation of HNK-1+ neuroblast-like cells in the CB, whose activation by hypoxia-induced neuronal activity leads to an increase in the number of neuronal cells in the organ, and therefore to physiological adaptation of the tissue to the persistence of the stimulus.
We are currently testing the functional role of HNK-1 in proliferation and maturation of CB neuroblast-like cells. HNK-1 is a membrane glycoprotein that has been classically described as a neural crest marker during development. More recently, this protein has been proposed to be involved in intercellular adhesion, cell migration and synaptic plasticity in the central nervous system (CNS). Since CB neuronal cells tend to form highly cohesive glomeruli, we hypothesize that HNK-1 is important for new neuronal cells to remain attached to the neuronal glomerulus, to be able to complete differentiation including the formation of synaptic contacts with sensory nerve fibers present in the neuronal glomerulus.
Understanding the physiology of the CB neurogenic niche is crucial not only to learn more about adult neurogenic niches but also to understand the physiopathology of the organ and to improve the use of this tissue for therapeutic purposes.

W-3207

EFFECTS OF EPIDERMAL GROWTH FACTOR ON NEURAL STEM CELLS IN JUVENILE, ADULT, AND OLD RATS AFTER POST-NATAL IRRADIATION

Olle R. Lindberg, Magdalena Rosinski, Georg Kuhn
Institute of Neuroscience and Physiology, University of Gothenburg, Gothenburg, Sweden

Ionizing irradiation is currently one of the primary experimental models for stem cell ablation. The young brain is particularly sensitive to irradiation, in part because of the high prevalence of dividing stem and progenitor cells. Irradiation treatment at a young age can permanently abolish the neural stem cells of the brain. The neurogenic niches of the subventricular zone (SVZ) and the dentate gyrus, not only suffer a reduction of the stem cell pool after irradiation, but the niche itself is permanently altered. With a decreased ability to support proliferation or migration of stem cells, irradiation-induced changes in the neurogenic niche contribute to long-term detrimental effects of irradiation. EGFR (epidermal growth factor receptor) signaling is one of the main characteristics of proliferative neural progenitor cells. EGFR stimulation via EGF infusion results in a drastic restructuring of the SVZ, including induction of extensive proliferation and migration of glial-like progenitors. We have previously described EGF-induced hyperproliferative polyp-formation and dysplastic changes in the SVZ.
The high proliferative capacity of EGF-responsive cells in the naïve SVZ makes them vulnerable to the initial cytotoxic effects of irradiation treatment. However, EGF stimulation via infusion could potentially aid in the recovery of the irradiated stem cell niches, short and long term.

In the current study we are investigating the effects of EGF infusion on the brain in young, adult and old rats after post-natal whole-brain irradiation. Animals received irradiation (8Gy) at post-natal day 9 (P9) and were treated with intracerebroventricular infusion of EGF for two weeks at P21, P80, and one year after irradiation. EGF infusion at P21 and P80 increased the volume of the irradiated SVZ compared to irradiated vehicle controls. The effects of EGF on the irradiated SVZ were overall greater after EGF infusion at P21 compared to P80. In addition, no differences in SVZ volume or proliferation were observed in vehicle-infused P80 animals, indicating a recovery of the SVZ after irradiation. One year after irradiation no significant differences in either proliferation or volume were observed between any of the groups. However, EGF-induced proliferative 'hot spots' in the form of polyps, protruding into the ventricular space, were still found in both sham-treated and irradiated animals. These polyps were observed after EGF infusion at all three different time points, including in animals receiving irradiation. The EGF-induced polyps were surprisingly similar phenotypically and in terms of mitotic activity. The ability of EGF to create a proliferative niche in the, to neurogenesis, unfavorable environments of the aged and the irradiated SVZ is surprising. It could help increase our knowledge on stem cell-niche interactions and how to aid the recovery of neurogenesis after irradiation, and possibly how to slow down the age-related decline in neurogenesis.

W-3208

PICK1 AND PKCα REGULATE JAGGED1 ACTIVATION TO CONTROL NEUROGENESIS

Robert Beattie[1], Antje Grabosch[2], Marion Rukavina[2], Verdon Taylor[1]

[1]Biomedicine, University of Basel, Basel, Switzerland, [2]Molecular Embryology, Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

During formation of the brain in mammals, neural stem cells (NSCs) transit through sequential periods of expansion, neurogenesis and gliogenesis. Notch ligands are expressed by differentiating progenitors and activate lateral inhibition signals through Notch, maintaining NSCs and blocking transcription of pro-neurogenic factors. Deletion of Delta or Notch from NSCs results in precocious neurogenesis and aberrant brain formation. However, Notch signaling has long been proposed to be bidirectional through ligands such as Jagged1 (Jag1) which is also a type I membrane protein. The molecular mechanisms controlling the transition from stem cell division, where Notch plays a maintenance role, to daughter cell differentiation are poorly understood. Through biochemical and transgenic analysis, we show that PICK1 and PKCα regulate proteolytic activation of the Notch ligand Jag1 to release its intracellular domain (ICD). Nuclear translocated Jag1ICD regulates neurogenesis during cortical development. We propose that Jag1 acts as a signal integrator to modulate neural progenitor fate and drive the onset of differentiation in the developing brain. This model provides a novel paradigm for reciprocal lateral signaling through a Notch ligand to modulate cell fate.

W-3211

LOSS OF DICKKOPF 1 RESTORES NEUROGENESIS IN OLD AGE AND COUNTERACTS COGNITIVE DECLINE

Désirée R. M. Seib[1], Nina S. Corsini[3], Kristina Ellwanger[2], Christian Plaas[4], Alvaro Mateos[1], Claudia Pitzer[4], Christof Niehrs[2], Tansu Celikel[5], Ana Martin-Villalba[1]

[A]Molecular Neurobiology, [B]Molecular Embryology, [1]DKFZ-ZMBH Alliance, German Cancer Research Center (DKFZ), Heidelberg, Germany, [2]Institute of Molecular Biotechnology (IMBA), Vienna, Austria, [3]Sygnis Bioscience, Heidelberg, Germany, [4]Laboratory of Neural Circuits and Plasticity, University of Southern California, Los Angeles, CA, United States

Adult mammalian neurogenesis takes place in two neurogenic niches in the brain, the subventricular zone of the lateral ventricles and the subgaranular zone of the dentate gyrus. The process of neurogenesis, including the birth of new progenitors, their neuronal differentiation, survival and finally their functional integration into the network, can be influenced by various intrinsic and extrinsic factors within the microenvironment. Specifically, ageing is one factor that leads to a decrease in adult neurogenesis. This age-related decrease in adult hippocampal neurogenesis has been associated with the decline of cognitive functions in the aging population. Although several pathways are known to regulate multiple aspects of adult neural stem cell biology, the molecular basis of the reduction of neurogenesis in the aging hippocampus remains unclear.
Wnt signals are important regulators of adult hippocampal neurogenesis. Here we show that expression of the Wnt antagonist Dickkopf-1 (Dkk1) increases with age in the dentate gyrus and that its loss enhances hippocampal neurogenesis. Conditional deletion of Dkk1 in adult neural stem cells increases their Wnt activity, leading to enhanced self-renewal and a higher production of immature neurons. This Wnt-expanded progenitor population subsequently differentiates into mature glutamatergic granule neurons with increased dendritic complexity.
We also find an increased number of Arc-positive dentate granule cells in Dkk1 mutants, which indicates an increased activity of the neuronal network. Mice that lack Dkk1 show enhanced spatial working memory and memory consolidation, whereby learning itself is not affected. Notably, the performance of middle-aged Dkk1-mutants was set back to the levels exhibited by control young animals. Dkk1 mutant mice also show reduced behavioral despair and increased hedonic behavior. In summary, our findings show that exclusive deletion of Dkk1 in adult neural stem cells increases their levels of Wnt activity and counteracts the age-related decrease in neurogenesis and its associated cognitive decline. Furthermore, it improves affective behavior in the general population.

W-3212

REPROGRAMMING FIBROBLASTS INTO NOCICEPTIVE NEURONS.

Brian Wainger[1], Elizabeth Buttermore[1], Julia Oliviera[1], Naomi Tsujimoto[2], Isaac Chiu[1], Justin Ichida[3], Lee Rubin[2], Kevin Eggan[2], Clifford Woolf[1]

[1]FM Kirby Neurobiology Center, Boston Children's Hospital, Boston, MA, United States, [2]Department of Stem Cell and Regenerative Biology, Harvard Stem Cell Institute, Cambridge, MA, United States, [3]Broad CIRM Center for Regenerative Medicine and Stem Cell Research, Keck School of Medicine of USC, Los Angeles, CA, United States

The study of pain in humans is confounded by subjective interpretation as well as discordance between severity of pain and pathology. Similarly, animal models and heterologous expression studies show marked limitations, and many drugs that appeared promising in animal studies ultimately failed in the clinic. The use of stem cell technology to develop nociceptors offers

a promising approach to modeling pain. Unlike many neuronal subtypes, nociceptors have a very well-defined and specific assortment of receptors and transducers. The most characteristic of these include the tetrodotoxin (TTX)-resistant sodium channel NaV1.8 and the capsaicin receptor, TrpV1. However, production of nociceptors has proven difficult, with little success using traditional morphogen-based approaches and the production of only a low percentage of capsaicin-responding cells using a recent small molecule-based screening approach (Chambers et al, 2012). We have taken a different technique, using forced transcription factor reprogramming of fibroblasts, as has been used to make generic and specific neuronal subpopulations (Vierbuchen et al, 2010; Son et al, 2011). Using TrpV1 or NaV1.8 Cre drivers to generate reporters in mouse embryonic fibroblasts, we have obtained efficient conversion from the fibroblasts into functional neurons that exhibit robust currents in response to capsaicin. The neurons also have TTX-resistant sodium currents with voltage and kinetic properties consistent with NaV1.8. We are currently performing additional characterization using immunohistochemistry, expression profiling, calcium imaging, and patch clamp. In the future, we hope to use the reprogramming technique to evaluate pain and neuropathy phenotypes and to screen for potential analgesics in human cells.

W-3213

IDENTIFICATION AND CHARACTERIZATION OF RETINAL GANGLION CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Jason S. Meyer, Manav Gupta, Akshayalakshmi Sridhar
Biology, Indiana University-Purdue University Indianapolis, Indianapolis, IN, United States

Derived from patient samples, human induced pluripotent stem cells (hiPSCs) have the potential to differentiate into any cell type of the body, providing a unique tool for cell replacement, disease modeling, and drug screening. To serve in this capacity, however, hiPSCs must be directed to properly differentiate to the cell type of interest. We have previously demonstrated the ability to differentiate hiPSCs to a retinal lineage, whereupon retinal photoreceptor and retinal pigment epithelium cells were the most abundant cell types produced. The ability to derive retinal ganglion cells (RGCs) from hiPSCs would not only serve as a novel model of human retinogenesis, but would also have profound implications for diseases such as glaucoma or other optic neuropathies. In the current study, we characterize the ability of hiPSCs to generate RGC phenotypes, including those cells expressing the RGC-specific transcription factors Brn3 and Islet-1. Furthermore, treatment of these cells with intrinsic and/or extrinsic factors known to influence the development of specific retinal cell types caused enhanced RGC specification from a more primitive retinal progenitor cell fate. Thus, the data presented within this study demonstrates that under the appropriate conditions, hiPSCs can serve as a reliable source of patient-derived RGCs. These results allow for future studies in which these hiPSC-derived cells may be utilized for studies of a variety of optic neuropathies afflicting the retinal ganglion cell population.

W-3214

IN VITRO MODELS OF MEDIUM SPINY AND VON ECONOMO NEURON DYSFUNCTION IN FRONTOTEMPORAL DEMENTIA

Kelly M. Haston[1], Hengameh Zahed[2], William W. Seeley[4], Michael C. Oldham[5], Steven Finkbeiner[3]
[A]Roddenberry Center for Stem Cell Biology and Medicine and Keck Program in Brain Cell Engineering, [B]Gladstone Institute of Neurological Disease, [C]Taube-Koret Center for Huntington's Disease Research and the Hellman Family Foundation Program, [1]UCSF Gladstone Institutes, San Francico, CA, United States, [2]The Selective Vulnerability Research Laboratory, UCSF Memory and Aging Center, San Francico, CA, United States, [3]Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, UCSF, San Francico, CA, United States

Frontotemporal dementia (FTD) is the second most common form of early onset dementia after Alzheimer's disease, and is characterized by degeneration of the frontal lobe and other brain regions. FTD has a heterogeneous clinical presentation, including changes in behavior, language, and movement. Large-scale brain networks are affec-

ted, with atrophy most prevalent in network-associated regions. Specific neurons from these regions are selectively vulnerable: in particular, von Economo neurons (VENs), large CTIP2-positive glutamatergic neurons found in layer Vb of the frontoinsula and anterior cingulate cortex. Furthermore, a 25% decrease in striatal volumes relative to healthy controls has also been reported, and the vulnerable population in this region is unknown. We hypothesize that medium spiny neurons (MSNs), GABAergic DARPP32-positive projection neurons with dense spines on their dendrites, which account for >90% of the neurons in the striatum are the afflicted population. The goal of this work is to develop direct reprogramming protocols to derive human in vitro models of FTD in two neuronal subtypes potentially affected in FTD, MSNs and VENs. To accomplish this we have used novel network-level transcriptome analysis from the anterior cingulate cortex, frontoinsula, and striatum to elucidate co-expression modules and "instructive factors" that will drive the in vitro development of MSNs and VENs. We introduce the "instructive factors" into human fibroblasts (FB) or iPSC-derived neural precursor cells (NPCs) derived from both healthy controls and FTD patients. Additional tools include mouse embryonic fibroblasts (MEFs) isolated from a double transgenic mouse model expressing reporters driven by promoters of either the dopamine D1 and D2 receptor, to indicate specific MSN sub-populations. Direct reprogramming of these MEFs will allow us to detect whether different combinations of instructive factors alter the ratio of the derived D1 to D2 expressing MSNs. Analysis includes morphology and immunofluorescence of the neuronal marker MAP2, along with other MSN and VEN specific proteins, to identify successfully reprogrammed cells. However, in collaboration with Sigma Aldrich, we are also developing human iPSC lines with Zinc Finger Nuclease-mediated insertion of fluorescent reporters driven by promoters for genes highly expressed in VENS and MSNs, such DISC1 and DAPRP-32 to allow selection of reprogrammed cells. Development of these reporter lines will allow us to use FACS or laser capture to isolate individual neurons and perform single cell gene expression analysis to determine if the reprogrammed cells are expressing appropriate MSN and VEN gene profiles. Ultimately, using novel robotic microscopy technology, we will perform longitudinal single-cell survival analysis, which will uncover differences in the ability to derive and maintain MSNs and VENs in control and FTD patient cells. Preliminary data suggest overexpression of multiple VEN factors in control FB and NPCs generates the distinctive VEN spindle morphology in Map2-positive cells, while overexpression of MSN factors promotes induced neurons that are both single and double positive for the dopamine D1 and D2 receptors.

W-3215

MODIFICATIONS IN NEUROSPHERE CULTURE CONDITIONS INFLUENCE NEURONAL DIFFERENTIATION OF CELLS FROM MEDIAL GANGLIONIC EMINENCE

Simone A. Romariz[1], Daisyléia Souza Paiva[1], Maria Elisa Calcagnotto[2], Gabriela Filoso Barnabé[3], Beatriz Monteiro Longo[1]

[1]Neurophysiology, Universidade Federal de São Paulo, São Paulo, Brazil, [2]Biochemistry, Universidade Federal do Rio Grande do Sul, São Paulo, Brazil, [3]Ludwig Institute for Cancer Research, São Paulo, Brazil

Neural stem cells (NSC) from the medial ganglionic eminence (MGE) of the developing brain are responsible for the origin of most inhibitory interneurons in the cortex and hippocampus of the mature brain. *In vitro* these cells preserve the ability to differentiate into inhibitory interneurons. NSC can be cultivated as neurospheres, cell aggregates in suspension, which provide expansion of neural progenitors that differentiate into three cell types: neurons, astrocytes and oligodendrocytes, characterizing an attractive method to study the potential and applicability of NSC for cell replacement therapy in neurological diseases. Here, we tested whether modifications in culture conditions (in the presence of retinoic acid and / or removal of growth factors) influence neuronal differentiation and specification of MGE-derived NSC *in vitro*, enhancing the population of inhibitory interneurons.
MGE from rat embryos (E14) were dissected and the cells were grown as neurospheres in four distinct culture conditions: 1) in the presence of epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2) (group E/F); 2) with EGF/FGF-2 plus retinoic acid (RA) (group E/F+RA); 3) only RA (group mitogens free MF+RA) and 4) absence of either EGF/FGF-2 and RA (group MF). In all conditions, the culture medium DMEM/F12, L-glutamine, N2 supplement and antibiotic were present. After 7 days in these culture conditions, neurospheres were plated on an adherent substrate to differentiate for 10 days. The pattern of differentiation was analyzed by immunofluorescence and

flow cytometry using specific markers for neural progenitors (Nestin), astrocytes (GFAP), neurons ([[Unsupported Character - Symbol Font ]]tubIII) and specific inhibitory neurons (Neuropeptide Y – NPY and Parvalbumine - PV).
Neurospheres that grew in the absence of the growth factors EGF and FGF-2 (MF) were smaller when compared to cells cultured in media rich in mitogenic factors (E/F). Also, data from flow cytometry and immunofluorescence indicated that neurospheres cultured only with RA (MF+RA), showed a significantly larger proportion of neurons (23,35% of β-tubIII-positive cells) when compared to the group E/F (2,96%). On the other hand, the media containing EGF/FGF-2 plus RA (E/F+RA) impaired the differentiation of cells, because it increased the number of undifferentiaded neural precursors (40,44% of Nestin-positive cells versus 22,17% E/F, 15,99% MF+RA and 9,39%% MF). Among the inhibitory neuron markers analyzed, the
presence of PV-positive neurons (23,84 %) was higher compared with NPY (43,36%), and a greater percentage of inhibitory neurons was found in the group MF.
Our data suggest that variations in media composition were able to modify the pattern of neuronal differentiation *in vitro*. The absence of EGF and FGF-2 and the addition of retinoic acid affects the neurosphere growing potential and the cell fate by increasing the population of neurons and inhibitory neurons. Cells cultured under specific conditions may be used, in the future, for transplantation into the nervous system aiming diseases that need neuronal replacement.

W-3216

RELATIVE AND COMBINATORIAL EFFECTS OF NICHE-DERIVED FACTORS ON THE BEHAVIOR OF ADULT FOREBRAIN NEURAL PRECURSORS

Sandra E. Joppe, Anne Aumont, Karl J. Fernandes
Universite de Montreal, Montreal, QC, Canada

A complex issue in the study of adult neurogenesis is how neural precursor cells are regulated by the variety of molecules present within their local niche. The subventricular zone of the lateral ventricles is the principal neurogenic niche of the forebrain, and contains a large number of regulatory molecules, such as EGF, FGF, BMP, PDGF, VEGF, SHH and Notch/Delta families. These niche-derived factors have typically been studied individually, and so their relative and combinatorial effects on neural precursor cell behaviors (proliferation, differentiation and survival) remain poorly understood. Here, our objective was to perform a side-by-side comparison of the above niche-derived factors on neural precursor cell cultures isolated from the forebrain of adult mice. First, neurosphere suspension cultures were treated during neurosphere formation and analyzed to assess the effects of individual niche-derived factors on neural precursor cell proliferation and survival. Second, adherent cultures of dissociated neurospheres were treated with these individual factors and analyzed by Western blotting to measure changes in neural precursor cells differentiation. Third, multi-passage experiments were performed to examine long-term effects on neural precursor cell expansion and lineage specification. Finally, combinations of niche-derived factors are currently being tested to determine whether particular factors have additive or dominant effects on neural precursor cell behavior. Preliminary results reveal widely differing impacts of niche-derived factors on the biological properties of cultured neural precursors, and indicate that that particular factors can exert additive or dominant effects with used in combination. We are currently complementing our in vitro findings using in vivo approaches. These experiments will help reveal which niche-derived factors are the most influential regulators of neural precursor cells behavior.

W-3217

THE SPECIFICATION AND MATURATION OF NOCICEPTIVE NEURONS FROM HUMAN EMBRYONIC STEM CELLS

Erin M. Boisvert[1], Shawn E. Hallowell[3], Sandra J. Engle[3], Xue-Jun Li[2]
[A]Genetics and Developmental Biology, [B]Neuroscience, [1]Univ. of Connecticut Health Ctr., Farmington, CT, United States, [2]Genetically Modified Models RCOE, Pfizer Worldwide Research and Development, Groton, CT, United States

It has been estimated that in America alone chronic pain directly affects more than 116 million adults. Nociceptive neurons play an essential role in the sensation of pain as they are responsible for transmitting painful stimuli to the nervous system. However, a major limitation in studies to determine the mechanisms of action as well as potential therapeutics in the human system has been a lack of nociceptive neurons to evaluate. Based on our successful generation of neural lineage cells and spinal progenitors from human embryonic stem cells (hESCs), we investigated the specification and maturation of nociceptive neurons from hESCs by establishing a chemically defined system. Human ESCs were first differentiated into the neural lineage using our differentiation paradigm as previously described. The addition of retinoic acid and bone morphogen factor 4 (BMP4) at specific time points and concentrations yielded a high population of neural crest progenitor cells (AP2a+, P75+). These progenitors were then dissociated and plated onto coated coverslips for terminal differentiation in the presence of neurotrophic factors. Several weeks after differentiation, nociceptive neurons (TrkA+) were generated in the cultures. These mature neurons also stained positive for sodium channel markers (Nav 1.7, Nav1.8) as well as P2X3 which plays a role in the peripheral response to pain. Furthermore, the overexpression of Neurogenin 1 (Ngn1) a key player in the decision of a neural crest progenitor cell to differentiate into a nociceptive neuron, promoted the expression of sensory neuron related genes (Brn3a, TrkA) in the cultures. The optimal time window for Ngn1 expression to generate the highest population of mature nociceptive neurons, as well as the functional capabilities of these cells is under investigation using our recently established Ngn1 inducible hESC system. Overall this study provides insight into the mechanisms underlying the specification of nociceptive neurons from hESCs and it generates a potential tool for high throughput screens of therapeutic agents for pain.

W-3218

MICROENVIRONMENTAL CHANGES REGULATE NOTCH SIGNALING PATHWAY IN SUBVENTRICLULAR ZONE NEURAL STEM CELLS IN PATHOLOGICAL CONDITIONS.

Manideep Chavali[1], Adan Aguirre[2]

[A]Material Sciences and Engineering, [B]Department of Pharmacological Science, [1]Stony Brook University, Stony Brook, NY, United States

In the normal brain, different cell types and signaling cues create a niche that instruct stem cells to proliferate, migrate and differentiate in order to maintain tissue homeostasis. The unique microenvironment and the cytoarchitecture of subventricular zone (SVZ) maintain neural stem cells (NSCs; type B) and transit amplifying progenitor cells (NPCs; type C) pool size in the adult mammalian brain. Under normal conditions, basal processes of type B cells are in contact with blood vessels and slowly divide to generate type C cells. Thus, the niche sustains cellular homeostasis, but in pathological conditions microenvironmental changes can profoundly affect stem cell behavior, exposing the cells to factors they would not normally encounter. We hypothesized that these factors disturb the natural balance, thus neural stem cells adapt to new conditions in what is believed to be an effort to stop ongoing damage and promote repair. While signaling pathways and related effects of the adult SVZ-niche remain fairly well understood, pathways activated following injury and resulting outcomes are not completely known. Identification of these signaling cues and their outcomes is crucial for developing cell-based strategies to promote functional repair from endogenous stem/progenitor cells. Using animal models of acute subcortical white matter (SCWM) demyelination, we demonstrate that during peak demyelination, Notch signaling in SVZ type B cells is downregulated and the stem cell numbers are decreased. The signaling molecules involved in this pathway were restored back to normal after remyelination. Intriguingly, we also found that in SVZ, vascular network expresses delta 1 (DLL1) ligand, which is required for the activation of notch signaling. During demyelination the number of type B cells contacting the blood vessels were reduced from the ependyma as compared to control conditions. Our data suggests that during demyelination, type B cells lose contact with blood vessels (BVs) and this leads to a loss of self renewal capacity; generating higher number of type C cells, which initiate the remylineation process. Currently we are investigating what signaling cues lead to this contact breakage and the expression of adhesion molecules involved in the anchorage of type B cells to BVs.

W-3221

TRANSFORMATION OF NEURAL STEM CELLS BY NOTCH1 IN VIVO

Sivaraman Natarajan[1], Yaochen Li[1], Emily Miller[1], Michael Taylor[2], Timothy Stearns[1], Roderick Bronson[3], Susan Ackerman[1], Jeong Yoon[4], Kyuson Yun[1]

[1]The Jackson Laboratory, Bar Harbor, ME, United States, [2]Division of Neurosurgery, Program in Developmental and Stem Cell Biology, Hospital for Sick Children, Toronto, ON, Canada, [3]Dana Farber Cancer Institute, Boston, Boston, MA, United States, [4]Maine Medical Center Research Institute, Scarborough, ME, United States

Notch signaling plays an important role in regulating survival, proliferation and maintenance of normal and cancer stem cells in the brain. For example, loss-of-function mutations in Notch pathway genes result in precocious depletion of neural stem cells in the mouse brain, and inhibition of the Notch pathway in neural stem cells and glioma stem cells blocks self-renewal of these cells in vitro. Activation of the Notch pathway results in localization of the intercellular domain of Notch receptors (NICD) to the nucleus, where it binds to transcriptional cofactors to activate many downstream target genes. While activation of the Notch pathway is observed in many human cancers, including brain cancer, it is unknown whether Notch1 activation is sufficient to cause brain tumor formation. To test whether Notch1 has oncogenic function in neural stem and progenitor cells in vivo, we expressed an activated form of NOTCH1 (N1ICD) in the developing mouse brain. N1ICD;GFAP-cre mice were viable but developed severe ataxia and seizures, and died by weaning age. Analysis of transgenic embryonic brains revealed that N1ICD expression induced p53-dependent apoptosis. When apoptosis was blocked by genetic deletion of p53, 30~40% of N1ICD;GFAP-cre;p53+/- and N1ICD;GFAP-cre;p53-/- mice developed spontaneous medulloblastomas. Interestingly, Notch1-induced medulloblastomas most closely resembled the sonic hedgehog (SHH) subgroup of human medulloblastoma. However, when compared to medulloblastomas induced by activation of the SHH pathway (Ptch+/-), Notch1-induced tumors expressed significantly higher levels of genes involved in translational control, chromatin structure, and cellular metabolism, suggesting differences in epigenetic mechanisms of cellular transformation by the two stem cell pathways. In summary, this study demonstrates that elevation of Notch1 activity in neural stem cells can cause cellular transformation when combined with other oncogenic events, such as p53 loss; however, the mechanism of transformation is not through stem cell expansion but rather through induction of DNA damage and epigenetic changes.

W-3222

MICROCEPHALY ASSOCIATED PROTEIN WDR62 REGULATES NEUROGENESIS THROUGH JNK1 IN THE DEVELOPING NEOCORTEX

Zhiheng Xu, Dan Xu, Feng Zhang
Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China

Mutations of WD40-repeat protein 62 (*WDR62*) have been identified recently to cause human MCPH (autosomal recessive primary microcephaly), a neurodevelopmental disorder characterized by decreased brain size. However, the underlying mechanism is unclear. Here, we investigated the function of WDR62 in brain development and the pathological role of *WDR62* mutations in MCPH. We used shRNAs to deplete WDR62 expression in neural progenitor cells (NPCs) via *in utero*
electroporation (*IUE*) in the brain and found that WDR62 knockdown leads to premature differentiation of NPCs. The defect can be rescued by wild-type human *WDR62* but not the five MCPH associated mutant and a mutant that is unable to interact with and activate JNK1. We explored the potential underlying mechanism by which WDR62 affects the coordinated proliferation and differentiation of NPCs. The presence of multiple spindle poles and centrosomes and disturbed mitotic orientation (increase of asymmetric divisions) in WDR62 knockdown NPCs indicates the deregulation of mitosis. In addition, the interaction between WDR62 and JNK1 is essential for WDR62's ability to activate the JNK signaling. JNK1 depletion also leads to premature NPC differentiation and over-expression of JNK1 can rescue WDR62 depletion-incurred different defects. Thus our findings indicate that WDR62 is required for the timing of neurogenesis via JNK1 and provide an insight into the molecular mechanisms underlying MCPH pathogenesis.

W-3223

NESTIN-EXPRESSING HAIR FOLLICLE STEM CELLS FORM THE WHISKER SENSORY NERVE IN LONG-TERM 3D CULTURE

Sumiyuki Mii[1], Jennifer Duong[1], Yasunori Tome[1], Aisada Uchugonova[2], Fang Liu[3], Yasuyuki Amoh[4], Norimitsu Saito[4], Kensei Katsuoka[4], **Robert M. Hoffman**[1]

[1]AntiCancer Inc., San Diego, CA, United States, [2]Department of Biophotonics and Laser Technology, Saarland University, Saarbruecken, Germany, [3]Department of Anatomy, Second Military Medical University, Shanghai, China, [4]Department of Dermatology, Kitasato University School of Medicine, Kanagawa, Japan

Nestin-expressing hair follicle stem cells can differentiate into neurons, Schwann cells, and other cell types. In the present study, vibrissa hair follicles, including their sensory nerve stump, were excised from transgenic mice in which the nestin promoter drives green fluorescent protein (ND-GFP), and were placed in 3D culture supported by Gelfoam®. The whisker nerve stump was regenerated via trafficking nestin-expressing stem cells originating in the bulge of the whisker in Gelfoam® histoculture. The growing nerve fibers had growth cones on their tips expressing F-actin, indicating they were growing axons. The growing whisker sensory nerve was highly enriched in ND-GFP cells which played a major role in its elongation and interaction with other nerves in 3D culture, including the sciatic nerve, the trigeminal nerve, and the trigeminal nerve ganglion. The results of the present report suggest a major function of the nestin-expressing stem cells in the hair follicle is for growth of the follicle sensory nerve. The results also demonstrate the use of Gelfoam® histoculture and hair follicle nestin-expressing stem cells for nerve regeneration.

W-3224

DEVELOPMENTAL CUES INDUCE THE DIFFERENTIATION OF NEURAL CREST DERIVED ORAL MUCOSA STEM CELLS INTO THERAPEUTIC DOPAMINERGIC LIKE NEURONS

Javier Ganz[1], Yael Barhum[1], Tali Ben Tzur[1], Ina Arie[2], Sammy Poor[3], Shareef Araidy[3], Sandu Pitaru[2], Daniel Offen[1]

[A]Neurosciences Laboratory, [B]Oral Biology Dept, [1]Tel Aviv University, Tel Aviv, Israel, [2]Oral & Maxillofacial Dept, Baruch Padeh Medical Center, Poria, Israel

The oral mucosa possesses a high regenerative capacity regardless of the individual's age. Recently, a unique neural crest-like stem cell population (hOMSC) was isolated from the lamina propria of the adult human oral mucosa. In development, neural crest cells are the embryonic origin of the oral mucosa lamina propria, which is formed by migrating neural crest cells from the midbrain in the 4-somite stage. hOMSC exhibit pluripotency and neural crest stem cell markers and differentiate into neuroectodermal lineages. This phenotype is not affected by the donor age. These findings and the midbrain-related lineage origin make the hOMSC population an attractive candidate for cell replacement in Parkinson's disease. The aim of this study was to assess hOMSC ability to differentiate into dopaminergic-like neurons in vitro in response to developmental cues and to test the capacity of this differentiated population to improve symptoms in the hemi-Parkinsonian rat model. The results indicate that hOMSC express constitutively a repertoire of neural and dopaminergic markers: tyrosine hydroxylase (TH), Lmx1a and Nurr1. Exposure of hOMSC to a new differentiation medium consisting of soluble factors known to induce dopaminergic differentiation, induced a neuronal-like morphology in the large majority of the cells, downregulation of stem cells markers and upregulation of the characteristic dopaminergic markers TH, Pitx3, DAT, Nurr1, Otx2 and Lmx1a/b. A significant increase in the dopamine release was observed in DA-differentiated hOMSC (hOMSC-DA). Transplantation of hOMSC-DA into the striatum of hemiparkinsonian rats improved their behavioral deficits as determined by amphetamine-induced rotational behavior, motor asymmetry and motor coordination tests. These improvements were accompanied by the presence of hOMSC-DA expressing TH and increased levels of dopamine in the transplanted hemispheres. These results demonstrate for the first time that soluble factors involved in dopaminergic

differentiation induce hOMSC differentiation into dopaminergic-like neurons in vitro that are capable of improving motor function in hemiparkinsonian rats following their transplantation in vivo.

W-3225

UTILISING PATIENT-DERIVED IPSCS TO UNCOVER CELLULAR PHENOTYPES IN PARKINSON'S DISEASE

Elizabeth M. Hartfield[1], Hugo J. Fernandes[1], Michiko Yamasaki-Mann[1], Helen Christian[2], Jane Vowles[1], Samuel Evetts[1], Kevin Talbot[1], William S. James[1], Michele Hu[1], Sally Cowley[1], Richard Wade-Martins[1]
[A]The Oxford Parkinson's Disease Centre, [B]Department of Physiology, Anatomy and Genetics, [1]University of Oxford, Oxford, United Kingdom

Human induced pluripotent stem cells (hiPSCs) offer the potential to study otherwise inaccessible cell types. Critical to this is the directed differentiation of iPSCs into functional cell lineages. This is of particular relevance to research into neurological disease, such as Parkinson's disease (PD) in which midbrain dopaminergic neurons degenerate during disease progression but are unobtainable until post-mortem. The Oxford Parkinson's Disease Centre has recruited ~900 subjects (PD patients and controls) into its clinical cohort, with a target to collect 2000 subjects over 5 years. We have identified patients carrying mutations in the Leucine-Rich Repeat Kinase 2 (LRRK2) and Glucocerebrosidase (GBA) genes, two of the most commonly mutated genes found in PD. Thus far, 20 LRRK2, 24 GBA, 16 idiopathic PD and 21 control iPSC lines have been generated.
We have differentiated hiPSC lines into midbrain dopaminergic neurons and performed a comprehensive characterisation to confirm dopaminergic functionality by demonstrating dopamine synthesis, release and re-uptake. The neuronal cultures include cells double-positive for tyrosine hydroxylase (TH) and G protein-activated inward rectifier potassium channel 2 (GIRK2), representative of the A9 population of substantia nigra pars compacta (SNc) neurons vulnerable in PD. We used a combination of electrophysiology and calcium imaging to characterise the maturation of the slow autonomous pace-making (2-5 Hz) and spontaneous synaptic activity typical of mature SNc dopaminergic neurons
We then sought to examine autophagy pathways in these neurons using a combination of Western blot, immunocytochemistry and electron microscopy (EM). Initial data indicate increased levels of the lysosomal protein LAMP1 in all PD genotypes and EM analysis in LRRK2 patients indicated an expanded lysosomal compartment, as compared to controls. Increased levels of LC3B were also observed in patient neurons, indicating an upregulation of autophagosome formation. GBA is a lysosomal enzyme and we have found a ~45% decrease in GBA activity in neurons from GBA mutation carriers and a ~20% decrease in GBA activity in idiopathic patient neurons, compared to controls.
hiPSC-derived dopamine neurons from PD patients have perturbations in the cellular pathways responsible for processing damaged proteins and organelles which may implicate a common lysosomal dysfunction in multiple PD aetiologies.

W-3226

NOVEL NEURAL INDUCTION METHOD FOR EFFICIENT GENERATION AND CHARACTERIZATION OF NEURAL STEM CELLS (NSC) DERIVED FROM PARKINSON'S DISEASE (PD) PATIENT SAMPLE IPSC LINES

Marian S. Piekarczyk[1], Tori Sampsell-Barron[1], Kun Bi[1], Spencer B. Hermanson[1], Connie S. Lebakken[1], Laurie J. Reichling[1], Ramya Sundararajan[2], Malini Vangipuram[2], Mohan Vemuri[3], Yiping Yan[3], J. William Langston[2], Birgitt Schuele[2]
[1]Primary and Stem Cell Systems, Life Technologies, Madison, WI, United States, [2]Parkinson's Institute and Clinical Center, Sunnyvale, CA, United States, [3]Primary and Stem Cell Systems, Life Technologies, Frederick, MD, United States

Parkinson's disease (PD) is one of the most common neurodegenerative disorders affecting a million people in the United States alone, with 50,000 Americans being diagnosed with PD each year. The absence of physiologically relevant cellular models for PD represents a major bottleneck for PD research. Novel models are urgently needed to

accelerate the discovery of disease mechanisms and drug targets and for screening purposes which could rapidly translate into a wide range of clinical and therapeutic applications. Patient-specific iPSC-derived cell types have become an attractive tool for disease modeling *in vitro*.
For neuronal differentiation, one commonly used approach is embryoid body (EB) formation followed by neural rosette isolation and expansion. This approach can generate neural stem cells (NSCs) which can differentiate into different neuronal cell types and glia and can be cryopreserved for further maturation. The current limitation is that the process is laborious, inefficient, and the cells usually need to be further purified. To overcome these limitations, we developed a novel neural induction method that allows for the generation of NSCs from iPSCs within 7 to 14 days without the need for EB formation. In this study, we differentiated 4 PD iPSC lines and 2 age-matched control lines into neural stem cells using a novel neural induction/expansion media to differentiate iPSC from an adherent monolayer on different matrices or feeder cells. We demonstrate that the generated NSCs are karyotypically normal, express known NSC markers: Nestin, Sox1 and Sox2. Furthermore, gene expression analysis distinguishes these NSCs from their parental iPSCs and fibroblasts, and clusters them together with control NSCs derived from H9 ESCs.
In summary, the novel neural induction media allows for efficient generation of NSCs from PD patient sample iPSCs and has the potential for large scale NSC generation to be utilized for high throughput/high content screening and drug discovery.

W-3227

INVESTIGATING THE CONTRIBUTION OF INFLAMMATION TO IDIOPATHIC PARKINSON'S DISEASE USING HUMAN PLURIPOTENT STEM CELLS

Leah Boyer[1], Christopher Tse[1], Benjamin Campbell[1], Beate Winner[2], Zacharias Kohl[2], Kaoru Saijo[3], Christopher K. Glass[3], Juergen Winkler[2], Fred H. Gage[1]

[1]Salk Institute, La Jolla, CA, United States, [2]University Hospital Erlangen, Erlangen, Germany, [3]UC San Diego, La Jolla, CA, United States

Parkinson's Disease (PD) is the most common neurodegenerative movement disorder. Historically, PD has been considered a strictly neuronal disease; however, clinical observations and evidence from animal models suggest inflammation may contribute to disease progression. It remains controversial whether glial activation, and the resulting inflammatory cascade, is a result or a cause of neuronal death. Towards resolving this distinction, we have established cultures of human embryonic stem cell derived dopaminergic neurons, and primary human astrocytes and microglia. These neural cells are used to investigate the glial inflammatory response to extracellular insults, and the neuronal response to pro-inflammatory mediators. We have found that the glial derived pro-inflammatory response can be serially propagated between astrocytes and microglia following a single inflammatory insult. Our human cell based model also establishes the neurotoxic response to a glial derived increase pro-inflammatory cytokines, allowing us to study the inflammatory contribution to the pathological development of PD in a human system. The glial cells are also the foundation of a targeted compound-screening platform for agonists of orphan nuclear receptor Nurr1, whose dual role as both neuro-protective and anti-inflammatory renders it an ideal target for therapeutic intervention in PD. Furthermore, we are differentiating iPS cells from PD patients and controls into neural subtypes to investigate the contribution of inflammation to the progression of idiopathic forms of this degenerative disease. We aim to understand the role of inflammation in PD and identify key molecular events involved at early stages in PD to exploit as potential targets for therapeutic intervention.

W-3228

NOVEL ELECTROPHYSIOLOGICAL PHENOTYPE AND ION CHANNEL COMPOSITION IN IPS NEURONS FROM LRRK2 PARKINSON'S PATIENTS

Leonardo Rodriguez[1], Peter Miu[2], Sally Mak[1], Xin Jiang[2], Faneng Sun[1], Scott Marzano[1], Y. Anne Haung[1], Yanping Li[1], James W. Langston[1], Birgitt Schüle[1]

[1]The Parkinson's Institute, Sunnyvale, CA, United States, [2]Molecular Devices, Sunnyvale, CA, United States

Parkinson's disease (PD) is one of the most common neurodegenerative diseases of aging. There is no cure or effective way to slow disease progression, and the causes of the disease remain obscure. Our goal is to develop a functional experimental tool to study PD using induced pluripotent stem cell (iPSC)-derived dopaminergic (DA) neuron from a patient with LRRK2 parkinsonism. Here, we examine the differences in ion channel expression electrophysiological output, and dopamine release in LRRK2, p.G2019S iPS-derived neurons. We derived iPSCs from patients carrying the most common PD mutation in the leucine-rich repeat kinase 2 (LRRK2) gene, p.G2019S from patient skin fibroblasts using a 4-factor retroviral approach. All iPSCs were characterized for pluripotency, differentiation potential, and were karyotypically normal. Then, we differentiated iPSCs into neural progenitor cells (NPCs) using our published embryoid body approach and dual SMA inhibition. For final neuronal maturation, we differentiated NPCs into dopaminergic (DA) neurons using smoothened agonist (SAG) and fibroblast growth factor 8 (FGF8) for the first 10 days and then further differentiated cells in brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF) and cyclic adenosine monophosphate (dcAMP) for another 25 days.
For the *electrophysiological studies*, we analyzed the functional profiles of endogenously expressing ion channels, such as voltage-activated inward and outward currents in developing and mature neurons, to evaluate the functional similarity of these cells to primary neurons and used specific K ion channel blocker 4-aminopyramidine (4AP) and the Na ion channel blocker tetrodotoxin (TTX). This was performed on an automated patch-clamp robotic machine. For *assessment of channel composition*, we designed a custom ion channel qPCR array with 48 genes to look at the expression at three time points for DA neuron development, Na channels, K channels and Ca ion channels. Day 0 is the NSC stage before differentiation begins, day 10 is when we switch from our induction media to our maturation media, and the final time point is after full maturity is reached at day 35. Finally, we were assessing dopamine release in these LRRK2 mutant neurons by stimulation of the cells with high KCl and supernatant is measured for DA using HPLC.
Our data show differences between the LRRK2 mutant and control lines. We believe these differences are caused by the LRRK2 mutation. Thus we have a functional PD model-in-a-dish to facilitate drug discovery.

W-3231

DEVELOPMENT OF HIGH THROUGHPUT FUNCTIONAL SYNAPTIC ASSAYS IN NEURONS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Pascal Laeng[1], Chris M. Hempel[2], James J. Mann[2], Jeffrey R. Cottrell[2], David J. Gerber[3]
[A]Cellular Pharmacology, [B]MANTRA group, [1]Galenea Corp, Wakefield, MA, United States

Dysfunctions in synaptic transmission play a critical role in many central nervous system diseases. Phenotypic characterization of synaptic dysfunction in human neurons derived from induced pluripotent stem cells (iPSCs) has been limited by the low degree of functional synaptic maturation observed in these cells and the lack of sensitive, high throughput assays that enable efficient screening of synaptic function. We have previously described the development of a high-throughput screening system for identifying modulators of synaptic function (Hempel CM et al., 2011) that can be applied to address these limitations. We have used an upgraded version of the system to measure evoked functional synaptic activities in cultures of human neurons derived from iPSCs. We compared synaptic responses measured in human neuronal cultures to those of rat and mouse cortical neuronal cultures to estimate their degree of functional synaptic maturation and activity in response to various pharmacological agents.
Methods: Post-mitotic human neurons derived from iPSCs (hiPSC-neurons) and primary neuronal cultures isolated from rat or mouse embryonic cortex were seeded in 96-well plates. For analysis of the ability of neurons to initiate action potentials following field stimulation, neurons were loaded with Fluo-4. For analysis of presynaptic function, cultures were infected with an adeno-associated virus (AAV) used to deliver a synaptophysin-pHluorin fusion fluorescent reporter construct (sypHy). Cultures were analyzed between 2 and 8 weeks in vitro on the upgraded MANTRA™ (Multiwell Automated NeuroTRansmission Assay) system or on a fluorescence microscope imaging system. For both systems, fluorescence imaging was performed in parallel with field stimulation trains.

Results: We found that hiPSC-neurons exhibit expected neuronal and synaptic morphology and can be reliably transduced with AAV to express sypHy with no signs of cytotoxicity. Human iPSC-neurons showed robust Ca^{2+} responses to field stimulation trains with voltage thresholds similar to those of rat neurons, suggesting reliable generation of action potentials. The hiPSC-neurons at 3-8 weeks in vitro displayed measureable levels of evoked presynaptic activity on the MANTRA system. Evoked presynaptic responses in hiPSC-neurons show similar frequency-dependent characteristics to those of rodent neurons. Follow- up fluorescence microscopy analysis confirmed the synaptic localization of sypHy signals in hiPSC-neurons. Although presynaptic responses were lower in hiPSC-neurons than in rat primary neuronal cultures, several conditions were found to increase the responses in hiPSC-neurons. Treatment with a compound that modulates presynaptic function yielded similar effects in hiPSC-neurons and rodent primary neuronal cultures. Also, co-culturing hiPSC-neurons with glia resulted in a robust increase in presynaptic responses to field stimulation. Experiments are ongoing to examine whether human astrocytes derived from iPSC can also promote synaptic functionality of human neurons.
Conclusions: The high-throughput capacity of the MANTRA system can be used to quickly characterize the functional and pharmacological properties of synaptic activities in hiPSC-neurons derived from iPSC. Ultimately, our high throughput synaptic assays can be used to characterize synaptic abnormalities in neurons derived from patients and to screen for compounds that restore normal synaptic transmission.

W-3232

PATIENT-SPECIFIC IPSC-DERIVED NEURONAL CELL MODELS OF HUMAN TAUOPATHIES

M. Catarina Silva, Chialin Cheng, Surya Reis, Wen-Ning Zhao, Steven Sheridan, Tau Consortium, Stephen J. Haggarty
Department of Neurology, Center for Human Genetic Research, Massachusetts General Hospital & Harvard Medical School, Boston, MA, United States

Neurodegeneration is caused by progressive loss of neuronal structure and function in the brain, and has been shown in many cases to be strongly associated with the presence of potentially deleterious protein aggregates. Given the prevalence of neurodegenerative disorders, and lack of effective therapeutics, new approaches for early diagnostics and to identify disease-modifying therapeutic targets are required. Recent advances in human genetics have led to the discovery of genetic susceptibility factors in a growing number of neurodegenerative disorders, including the frontotemporal dementia-spectrum disorders (FTD-s) consisting mainly of FTD, progressive supranuclear palsy (PSP) and corticobasal syndrome. In the case of FTD-s, coding and non-coding genetic variation in the MAPT gene, which encodes the microtubule-associated protein tau, have been identified as the main cause. With the long-term goal of elucidating the molecular basis of tau-associated neurodegeneration in the context of specific FTD-s, we have begun characterizing disease-associated phenotypes using patient-specific induced pluripotent stem cell (iPSC) models. From these FTD-s iPSCs, we have derived expandable, self-renewing neural progenitor cell (NPC) lines and differentiated NPCs. The differentiation of these NPCs provides a system to investigate in cellulo pathology, with tau expression at physiological levels and in the appropriate genetic and cellular context, offering a unique opportunity to focus on the early events of pathogenesis. Our initial findings using western blot analysis and a panel of tau antibodies reveal that while tau levels are almost undetectable in proliferative NPC, expression increases over 5 weeks of differentiation with a predominant expression of 3R form of tau. In FTD-s cells, tau reached higher levels and at an earlier time-point relative to control cells, over the course of neuronal differentiation. Overall, our findings support the relevance of using patient-derived iPSCs and neuronal cells for investigating the molecular mechanisms of FTD-s. Characterization of tau post-translational modifications, aggregation and processing into lower molecular weight fragments is on-going. In addition, we are pursuing the development of high-content, image-based assays that can support high-throughput screening of chemical, RNA interference (RNAi), and ORFeome libraries for modifiers of tau phenotypes, including clearance through autophagy pathways. We anticipate that expansion of this iPSC-based platform will enable the discovery and validation of effective disease-modifying, targeted therapeutics, with relevance for a large group of tauopathies and other dementias, such as Alzheimer's disease.

W-3233

ADVANCING TRANSLATIONAL NEURAL REGENERATIVE MEDICINE BY USING PIG INDUCED PLURIPOTENT STEM CELLS

Amalia Gallegos Cardenas, Kai Wang, Erin T. Jordan, Rachel C. West, Franklin D. West, Jeong-Yeh Yang, Steven L. Stice
Animal and Dairy Science, University of Georgia, Athens, GA, United States

The generation of pig induced pluripotent stem cells (iPSCs) opened the possibility to evaluate allograft and autologous neural cell therapy as a viable option for human patients. However, it is necessary to demonstrate whether pig iPSCs are capable of in vitro neural differentiation similar to human iPSCs in order to perform in vitro and in vivo comparative studies and to determine optimum differentiation conditions for forming functional neural therapeutics. A recent report from our group demonstrated that pig iPSCs can differentiate in vitro into neuron and glial cells and were capable of in vitro directed differentiation into motoneurons in the presence of motoneuron signaling factors. Multiple labs have generated pig iPSCs that have been characterized using pluripotent markers such as SSEA4 and POUF51. However, correlations of pluripotent marker expression profiles among iPSCs lines and their neural differentiation potential has not been fully explored. Because neural rosettes (NR) are composed of neural stem cells, our goal was to demonstrate that NR from pig iPSCs can be generated, isolated, and expanded in vitro from multiple porcine iPSCs lines similar to human iPSCs and that the level of pluripotency in the starting porcine iPSCs population (POUF51 and SSEA4 expression) could influence NR development.
Three lines of pig iPSCs from different donors, M1 (porcine mesenchymal stem cells), F1 (porcine fibroblast cells) and one sub-line from F1 (SLF1) were cultured on matrigel coated plates in mTeSR1 medium and passaged every 3-4 days. For neural induction, pig iPSCs were disaggregated using dispase and plated. After 24 hours, cells were maintained in N2 media (77% DMEM/F12, 10 ng/ml bFGF and 1X N2) for 15 days. To evaluate the differentiation potential to neuron and glial cells, NR were isolated, expanded in vitro and cultured for three weeks in AB2 medium (AB2, 1X ANS and 2mM L-Glutamine). Immunostaining assays were performed to determine pluripotent (POU5F1 and SSEA4), tight junction (ZO1), neural epithelial (Pax6 and Sox1), neuron (Tuj1), astrocyte (GFAP), and oligodendrocyte (O4) marker expression.
In this study we showed that all pig iPSCs were negative for NR markers Pax6 and Sox1. However, Line F1 ($POU5F1^{high}$ and $SSEA4^{low}$) showed a high potential to form NR in comparison to the other 2 lines M1 ($POU5F1^{low}$ and $SSEA4^{low}$) and SLF1 ($POU5F1^{low}$ and $SSEA4^{high}$) upon differentiation. The NR immunocytochemistry results from Line F1 showed the presents of Pax6+ and Sox1- NR cells at day 9 post-neural induction and that ZO1 started to localize at the apical border of NR. At day 13, NR cells were Pax6+ and Sox1+, and ZO1 was localized to the lumen of NR. After isolation and culture in vitro, NR cells expressed transcription factor OTX2 through 2 passages, but was not detected in later passages. However, rosette cytoarchitecture was present up until passage 7 and were still Pax6+/Sox1+. NR at passage 2 were cryopreserved and upon thaw showed normal NR morphology and were Pax6+/Sox1+. To characterize the plasticity of NR, cells were differentiated. Tuj1 expression was predominant after differentiation indicating a bias towards a neuron phenotype. These results demonstrate that F1 pig iPSCs ($POUF51^{high}$ and $SSEA4^{low}$) have a high potential to form NR and neural differentiation parallels human iPSCs neurulation events. Porcine iPSCs should be considered as a large animal model for determining the safety and efficacy of human iPSCs neural cell therapies.

W-3234

ELUCIDATING THE DEVELOPMENTAL MATURITY OF PLURIPOTENT STEM CELL DERIVED NEURAL PROGENITOR CELLS

Kimberly Loo, Xavier Gaeta, Michaela Patterson, William E. Lowry
Molecular, Cellular and Developmental Biology, University of California, Los Angeles, Los Angeles, CA, United States

Human pluripotent stem cells (hPSCs) have the potential to differentiate into many cell types, yet it is not known how similar the process of PSC in vitro development reflects the in vivo development. Recent work from the Lowry lab has found that both human embryonic stem cells and human induced pluripotent stem cells make cells that

are more similar to cells found only very early in fetal development. From this work, the Lowry lab identified a set of 88 genes whose expression appears to distinguish mature tissue derived cells from those generated from hPSCs. Among some of the differences observed between hPSC progeny and their respective natural counterparts are genes thought to only be expressed in early embryos, such ¬¬as LIN28, DPPA4, and TCF7L1. Furthermore, a second set of "maturity/specification" genes fails to be properly induced during in vitro differentiation. Both findings suggest incomplete specification or maturity of the PSC-derivatives. We hypothesize that gene manipulation experiments will be able to bring PSC derivatives closer to their natural counterparts on a global transcriptome level.
In attempts to make PSCs that more accurately reflect their natural postnatal tissue derived counterparts, experiments were performed using lentivirus infections aimed to overexpress several maturation/specificity genes in PSC derived neural progenitor cells (NPCs). Real-time reverse-transcription PCR (qRT-PCR) was used to quantify gene expression levels at the RNA level, while immunofluorescence was used to quantify expression levels at the protein level. Further, a functional assay was performed to evaluate the effects of the gene manipulations by determining whether the cells have a higher propensity to generate neuronal or glial cells.

W-3235

MODELING HEREDITARY SPASTIC PARAPLEGIAS USING HUMAN PLURIPOTENT STEM CELLS.

Kyle Denton[1], Ling Lei[1], Jeremy Grenier[1], Craig Blackstone[2], Xue-Jun Li[1]
[1]Department of Neuroscience, University of Connecticut Health Center, Farmington, CT, United States, [2]Cell Biology Section, Neurogenetics Branch, National Institute of Neurological Disorders and Stroke, Bethesda, MD, United States

Hereditary spastic paraplegias (HSPs) are a heterogeneous group of genetic disorders that result in progressive lower limb spasticity due to a length-dependent degeneration of axons, most severely affecting corticospinal motor neurons. Affected neurons display "dying back" axonopathy, particularly in the spinal cord, resulting in lost lower motor neuron innervations which produce lower limb spasticity and weakness. Although there are 55 distinct genetic loci where mutations are associated with HSP, one form, SPG4, accounts for nearly 50% of all cases of HSP. Autosomal dominant SPG4 mutations affect the SPAST gene, which encodes the microtubule-severing ATPase spastin. The lack of a human neuronal model of SPG4 has led us to generate human induced pluripotent stem cells (iPSCs) from affected patients using both the Cre-excisable STEMCCA lentiviral system and an episomal reprogramming method, which avoids transgene integration. These iPSCs expressed the standard set of pluripotency markers and maintained a normal karyotype after many passages. Using a well-established differentiation protocol, we found that the HSP iPSCs were able to differentiate into telencephalic glutamatergic neurons with similar efficiency as control iPSCs. Importantly, we found that the SPG4 neurons displayed a significant increase in axonal swellings after 6 weeks of differentiation (SPG4 = 0.824±0.238 swellings per 100 µm axon vs. control = 0.016±0.014 swellings per 100 µm axon), which previous studies have shown to be caused by microtubule-based transport defects. In addition, these swellings stain strongly for mitochondria and tau, suggesting the accumulation of axonal transport cargoes in these regions. This finding is in agreement with two knock out mouse models that lack spastin expression, and provides evidence that these iPSCs can be used to model HSP in vitro. To support these findings we have also generated four Tet-On spastin-inducible H9 embryonic stem cell lines that allow the overexpression of several spastin isoforms fused to GFP, including an ATPase-defective form of spastin (K388R). Cells expressing K388R spastin display normal neural differentiation; however, the localization of the mutant spastin is perturbed at all stages of differentiation observed, with strong colocalization with microtubules. In cells expressing a mutant M1 spastin isoform, we observed a significant decrease in the percentage of motile mitochondria. Overall, this study provides insight into the usefulness of a human stem cell model of SPG4, which should prove valuable for future studies aimed at dissecting the mechanism of axonal degeneration in this neurodegenerative disease.

W-3236

EXOGENOUS ADULT MOUSE POST MORTEM NEURAL PRECURSORS PROMOTE FUNCTIONAL RECOVERY IN A MOUSE MODEL OF PARKINSON DISEASE AND DIFFERENTIATE IN TH-POSITIVE NEURONS

Stephana Carelli, Andrea Raspa, Toniella Giallongo, Anna Maria Di Giulio, Alfredo Gorio
Health Sciences, University of Milan, Milan, Italy

Parkinson's disease is the second most common neurodegenerative disease, after Alzheimer's disease, and the most common movement disorder. Drug treatment and deep brain stimulation can ameliorate symptoms, but the progressive degeneration of dopaminergic neurons in the substantia nigra eventually leads to severe motor dysfunction. The transplantation of stem cells has emerged as a promising approach to replace lost neurons in order to restore dopamine levels in the striatum and
reactivate functional circuits. Post Mortem Neural Precursor Cells (PM-NPCs) are a subclass of SVZ-derived neural progenitors, capable of surviving hours after donor death. The *in vitro* differentiation yields more
neurons (about 30-40%) compared to regular NPCs. Recently from a transgenic mouse strain expressing green fluorescent protein (GFP) under the promoter C of the ubiquitin gene (C57BL/6-Tg(UBC-GFP)30Scha/J) we isolated PM-NPCs-GFP, from mice at 6 hours after death (T6). The potential of PM-NPCs in terms of replacement therapy was investigated in a mouse model of Parkinson disease. The degeneration of dopaminergic neurons was obtained with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
(MPTP) at the dosage of 36 mg/kg intraperiteoneally. Then the lesion was
stabilized by a second injection (i.p.) of the drug at the dosage of 20 mg/kg. 1x 10^5 of PM-PCs-GFP were administered to C57/BL mice by stereotaxic
injection unilaterally in the striatum 3 days after the second MPTP administration. The effects of transplanted cells were determined by means of performance tests aimed at detecting behavioral improvements. In order to perform histology studies aimed at investigating the fate of transplanted precursors after transplantation, animals were perfused 2 weeks after transplantation. Our results show that animals treated with T6 GFP-PM-NPCs had a remarkable improvement of parameters measured
by means of both horizontal and vertical grid tests (wall time, forepaw fault
and time required to grab on the grids while turning and climbing down) starting with the third day after transplantation. These improvements were
very significant and the average values were close to control. This was maintained throughout 2 weeks of experimental observation. By means of immunofluorescence staining we observed that the majority of transplanted
T6 GFP-PM-NPCs were vital and able to migrate ventrally and caudally from the injection site lengths as far as 1000 microns into the striatum, and could reach the ipsilateral and contralateral substantia nigra pars compacta. Moreover, morphological analysis revealed that transplanted cells in the striatum can differentiate into dopaminergic (40%), cholinergic (40%), and gabaergic neurons (20%). These data suggest how these cells may represent a liable source for cellular therapy in neurodegenerative disorders such as Parkinson Disease

W-3237

GENERATION OF AN INDUCIBLE MODEL OF STRIATAL ENRICHED PHOSPHATASE EXPRESSION IN HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED NEURONS

Sandra J. Engle[1], Jeffrey Stock[1], Thao Nguyen[2], Thomas Lanz[2]
[1]Pharmacokinetics, Dynamics and Metabolism, Pfizer, Groton, CT, United States, [2]Neuroscience Research Unit, Pfizer, Cambridge, MA, United States

Striatal enriched phosphatase (STEP, PTPN5) is a brain-enriched tyrosine phosphatase. Activation of STEP promotes the internalization of NMDA and AMPA receptors and the inactivation of ERK, p38, Pyk2 and Fyn, key molecules involved in synaptic strengthening and memory formation. STEP knock-out mice show increased phosphorylation of NMDA and AMPA receptor subunits as well as ERK and enhanced performance in hippocampal-dependent learning and memory tasks. Dysregulation of STEP has been associated with neurological disorders including schizophrenia, Alzheimer's disease, Huntington's disease and Fragile X. It has been suggested that inhibition of STEP may mitigate

symptoms associated with these disorders. To facilitate the study of STEP in neuronal function, we have generated human induced pluripotent stem cells (hiPSC) carrying a loxP-flanked target site in the AAVS1 locus (PPP1R12c). Recombinase-mediated cassette exchange was used to introduce a tet-inducible STEP containing a point mutation in the catalytic domain (C300S). $STEP_{(C300S)}$ continues to bind substrates but, lacking enzymatic activity, cannot dephosphorylate and disengage them. The “trapped” substrates can be immunoprecipitated and evaluated in an effort to identify novel STEP substrates and to determine how these substrates change with differentiation from neural progenitors to mature neurons.

W-3238

FIBRIN-BASED NICHE SUPPORTS IN VITRO DIFFERENTIATION OF NEURAL PROGENITORS FROM HUMAN PERIPHERAL BLOOD AND IN VIVO SURVIVAL OF TRANSPLANTED CELLS IN RATS

Tara S, Lissy K. Krishnan
Thrombosis Research Unit, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Poojappura,Trivandrum, India

Adult stem cells are being extensively studied as autologous source in regenerative medicine. Rare presence of NPC among the heterogenous and multipotent monocyte population necessitates further commitment to neural lineage and expansion by *in vitro* culture to obtain sufficient numbers of pure NPCs population for transplantation which may
be a promising strategy for the treatment of spinal cord injury (SCI). There
are challenges associated with cell transplantation including poor cell homing and survival at the injured site due to unfavorable mileu which
necessitates a good cell carrier for successful transplantation. The hypothesis of this study is that a fibrin-based biomimetic niche may support *in vitro* proliferation and lineage commitment of circulating NPCs, and the same may act as promising cell carrier for successful transplantation.
After experimenting with different concentrations of matrix molecules, the selected composition showed induction of human and/or rat NPCs to
neurons. The essential niche components identified were: adhesive proteins like fibrin, fibronectin, laminin and gelatin; growth factors in hypothalamus extract and platelet growth factor; hyaluronic acid and the medium supplemented with fetal calf serum, growth factors, retinoic acid and potassium chloride (KCl). The peripheral blood mononuclear cells (PBMNCs) were isolated from human and/or rat blood (Inbred Wistar strains) and the non plastic-adherent cells were cultured on the composed matrix for 10-15 days. Cells were analyzed at different time intervals for the expression and distribution of neural specific markers using immuno cyto chemistry. For *in vivo* experiments, contusion type SCI model was created using an impactor with ~150 dyne cm^{-2} force. The fluorescent tagged lineage committed cells (~$75x10^2$/site) mixed with fibrinogen composite was clotted at the site of injury using thrombin and cells suspended in culture medium was delivered in control sites. Basso, Beattie and Bresnahan (BBB) scoring was done weekly to evaluate the locomotor recovery and fluorescent imaging to visualise cell survival. Tissue sections were collected after 1 week and 4 weeks after transplantation for histological analysis.
Neural-like cells (NLCs) were observed in the standardized niche in human PBMNC culture from day5 but for rat PBMNC, NLCs were seen by day 10. The cells expressed neural progenitor marker nestin and the intermediate neural marker beta-tubulin3. Synaptophysin was expressed by a low percentage of cells at day 14. A small percentage of the cells were also positive for Map-2, a mature neuronal marker and GFAP, a glial marker. *In vitro* proliferation studies indicated that these NLCs were non-proliferative. The lineage commitment was independent of the presence of retinoic acid. Microscopically, cell to cell communication was observed to be improved upon KCl addition. Ninety percent of animals showed clinical signs of SCI, and improvement in BBB score was evident in most animals. Survival of transplanted cells was significant in test animals as compared to controls. Histological sections of the tissue collected from injured site at 1 week was comparable to that collected at 4 weeks, suggesting the prolonged survival but poor proliferation of transplanted cells.

Conclusion: Fibrin based niche supported *in vitro* differentiation of NPC from PBMNC and *in vivo* homing and survival of transplanted NLCs. So the bio mimetic fibrin matrix acts as a good cell delivery vehicle for SCI.

W-3241

MECP2E1 ISOFORM MUTATION CAUSES SYNAPTIC DYSFUNCTION IN RETT SYNDROME PATIENT IPSC DERIVED NEURONS

James Ellis, Ugljesa Djuric, Aaron Y.L. Cheung, Wenbo Zhang, Alina Piekna, Jason Hendry, P. Joel Ross, Peter Pasceri, Dae-Sung Kim, Michael Salter
Hospital for Sick Children, Toronto, ON, Canada

Rett Syndrome (RTT) is caused primarily by mutations in the X-linked MECP2 gene that affect two alternative transcripts encoding distinct MECP2e1 and MECP2e2 proteins. MECP2e1 is more highly expressed in the brain and rare patient mutations that affect only MECP2e1 protein have been described, but the cellular consequences of MECP2e1 mutation are unknown. We report the first MECP2e1-specific mutant cellular disease model by generating induced Pluripotent Stem Cells (iPSCs) from a RTT patient with a heterozygous 11bp deletion in exon 1.
We isolated four RTTe1-iPSC lines from this patient and differentiated them into neurons for phenotyping. The iPSC and neurons derived from them retain an inactive X-chromosome and express only the mutant MECP2 allele, indicating that MECP2 is not subject to erosion of X-chromosome inactivation over time as the iPSCs are passaged. Single cell Fluidigm expression arrays demonstrate that roughly 80% of the differentiated cells are mature neurons of predominantly cortical origin, and that MECP2e1 mutation does not affect neuronal fate choices in comparison to control neurons. Efficient differentiation required modified protocols for each line but consistently revealed a reduced soma size. These RTTe1 neurons exhibit subtle expression changes to a subset of MECP2 target genes. Infection of iPSC derived neural progenitor cells with a MECP2e1 isoform-specific lentivirus vector under the control of an internal mouse Mecp2 promoter rescues the soma size defect in a cell autonomous manner. Thus MECP2e1 mutation causes cell morphology and gene expression changes that may affect neuronal function.
We performed whole-cell patch-clamp recordings in RTTe1 and control neurons to investigate the cellular basis of RTT phenotypes. Mutant neurons had higher input resistance and exhibited decreased numbers of action potentials evoked by injecting depolarizing currents. Evoked action potentials in mutant neurons also had smaller amplitude and prolonged half duration that may be attributed to a significant decrease in voltage-gated Na+ currents. Furthermore, RTTe1 neurons had dysfunctional synaptic activity with decreases in both frequency and amplitude of miniature excitatory postsynaptic currents. Our findings demonstrate that MECP2e1 mutation causes dysfunction of Na+ channels and synaptic activity in addition to a cell autonomous soma size defect. These observations highlight that lack of MECP2e1 alone causes many RTT phenotypes in human neurons.

W-3242

RHOA REPRESSES PROLIFERATION IN THE CEREBELLUM AND IS A POTENTIAL TUMOUR SUPPRESSOR IN MEDULLOBLASTOMA FORMATION.

Dominik Herzog[1], Joao B. Relvas[2], Ueli Suter[3], Verdon Taylor[1]
[1]Department of Biomedicine, University Basel, Basel, Switzerland, [2]Glial Cell Biology, IBMC, Porto, Portugal, [3]Institute of Cell Biology, ETH, Zürich, Switzerland

We and others have recently shown an important role for the small GTPase RhoA in regulating proliferation and apical anchoring of neural stem cells in the developing mouse nervous system. The expression of RhoA negatively correlates with the grade of malignancy in gliomas and RhoA has been described to influence proliferation and migration in different glioma cell lines. However, the involvement of RhoA in postnatal cerebellar development and cerebellar tumour formation has not been addressed to date. We found that conditional ablation of RhoA from Nestin::Cre expressing radial glia resulted in viable mice that displayed disturbed cerebellar structure and postnatal hyperplasias that resemble early stage medulloblastomas. These RhoA conditional knockout (cKO) mice have increased proliferation in the external germinal layer (EGL) of the cerebellum as well as in granule cell precursor

(GCP) primary cell cultures in vitro supporting a cell autonomous function for RhoA. In the EGL of cKO mice the hyper-proliferation is accompanied with increased cell numbers resulting in tumour-like growths on the surface of the cerebellum. Furthermore mitotic GCP are not restricted to the outer EGL and invade the meningeal space forming ectopic hyperplastic tissue. Although proliferating cells are observed in these ectopic areas of EGL in the cKO, at ages where the EGL and proliferation in wild type mice is no longer present, the mutant cells are not transformed as they exit the cell-cycle and differentiate without forming tumours. In primary cell culture, GCPs from cKO mice have an increased basal proliferation rate in the absence of the mitogen Shh and this basal proliferation is insensitive to Shh inhibitors. In addition, the RhoA-deficient GCPs display enhanced responsiveness to Shh in vitro. We have preliminary evidence that the effect of RhoA to repress GCPs proliferation and regulate Shh activity is, at least partially, via regulation of ROCK. Taken together, RhoA represses proliferation in the cerebellum potentially by interacting with the Shh signalling pathway. Loss of Rho/ROCK signalling in the cerebellum leads to pre-neoplastic transformation of GCP which may make them more susceptible to medulloblastoma formation showing a potential role for RhoA as a tumour suppressor.

W-3243

SENSORY NEURON TRAJECTORIES ARE REGULATED BY AN RNA-BINDING PROTEIN THAT MODIFIES EXPRESSION OF A NEUROTROPHIN

Shinsuke Shibata, Hideyuki Okano
Department of Physiology, Keio University, School of Medicine, Tokyo, Japan

Segregation of touch and pain sensation pathways into the spinal cord is regulated to assure no cross-talk but it is not known how. Several lines of evidence support the idea that control of the release of neurotrophic factors is important for establishing normal trajectories for sensory axons. Here we demonstrate that the developmental release of one such factor is controlled by the RNA-binding protein Musashi2 (Msi2), known as one of the stem cell markers. This transient up-regulation by Msi2 plays a critical role in spinal mechanoreceptive network projection because the major msi2 knockout mice phenotype is hypersensitivity to touch sensation due to abnormal projection of mechanoreceptive axons into the pain receptive area. Thus, appropriate recognition of touch and pain as distinct sensations requires the embryonic surge of a neurotrophic factor under post transcriptional control of a RBP. We propose a novel regulatory mechanism for the segregation of sensory perception.

W-3244

ESTABLISHING AN IN-VITRO HUMAN SCHIZOPHRENIA MODEL FOR THE ANALYSIS OF DISEASE-ASSOCIATED NEURONAL SUBTYPES

Ralda Nehme[1], Ali Hussain[2], Karlijn van der Ven[3], Jurgen Del-Favero[3], Rolf Adolfsson[4], Annelie Nordin[4], Roy Perlis[5], Christine T. Finn[7], Raju Kucherlapati[8], Stephen J. Haggarty[6]
[1]Stanley Center for Psychiatric Research, Broad Institute of Harvard and MIT, Cambridge, MA, United States, [2]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States, [3]Department of Molecular Genetics, VIB, Flanders, Belgium, [4]Faculty of Medicine, Department of Clinical Sciences, Psychiatry, Umea University, Umea, Sweden, [A]Psychiatry Center for Experimental Drugs and Diagnostics, [B]Department of Neurology, [5]Harvard Medical School and Massachusetts General Hospital, Boston, MA, United States, [6]Psychiatry, Dartmouth-Hitchcock Medical Center, Lebanon, NH, United States, [7]Department of Genetics, Harvard Medical School, Boston, MA, United States

Schizophrenia is a disabling neuropsychiatric disorder that affects 1% of the world population. Despite its high prevalence, significant financial burden and high heritability (80%), the cellular and molecular mechanisms underlying schizophrenia are poorly understood. The strongest known risk factor for schizophrenia is a heterozygous deletion at human chromosomal locus 22q11.2 (22q11.2 deletion syndrome, 22qDS), affecting a region containing approximately 60 genes, many which regulate brain development.

Evidence from several sources such as brain imaging analyses and human post-mortem studies suggest that schizophrenia is a disorder of cortical neuron dysfunction, in particular layer III pyramidal projection neurons, characterized by thinning of the cerebral cortex and reduced dendritic spine density. Furthermore, genome-wide association studies (GWAS) implicate several genes involved in synaptic structure and function. Strikingly, mice carrying a deficiency in the region orthologous to 22q (mouse chromosome 16) exhibit defective neuronal differentiation and network formation. However, human prefrontal cortex differs enormously from the mouse, underscoring the need for human cellular models. Thus, we chose to study schizophrenia by developing and analyzing neurons and neural networks in cells from individuals with 22qDS. In order to generate a human in vitro system to recapitulate neuronal development, we obtained skin fibroblast from three patients with 22q11.2 deletion syndrome, and three unaffected individuals. We used the non-integrating Sendai virus to generate multiple induced pluripotent stem cell (iPSC) lines from each 22qDS and control fibroblast line. We validated at least three iPSC clones per individual. To test the hypothesis that 22qDS schizophrenia patients have defects in layer III pyramidal neurons, we are currently deriving layer III pyramidal neurons from both the 22qDS and the control iPSC lines. We are developing platforms to validate the identity of the generated neurons at a single cell level to determine their expression profile, and to evaluate their capacity to form functional synapses.
Our system will enable the cellular and molecular analysis of a neuronal cell type highly associated with schizophrenia, and sets the stage for the development of analogous frameworks for the derivation and examination of additional neuronal subtypes associated with schizophrenia and other psychiatric disorders.

W-3245

DIRECT DERIVATION AND PROSPECTIVE ISOLATION OF SCHWANN CELL PRECURSORS FROM HUMAN PLURIPOTENT STEM CELLS

Bipasha Mukherjee-Clavin[1], Soojung Shin[2], Gabsang Lee[1]
[1]Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2]Life Technologies Corporation, Frederick, MD, United States

Introduction: Genetic and acquired peripheral neuropathies are a significant source of disability, yet few effective treatments are available. Because of the role Schwann cells play in myelination and neuroregeneration, transplantation of Schwann cells or their precursor may offer a potential therapy in a subset of neuropathies. Here we report the derivation of Schwann cell precursors from human pluripotent stem cells. Method: A previously reported protocol for neuronal differentiation also yields Schwann cell precursors in close apposition to the neurons. Using dual-SMAD inhibition (with LDN-193189 and SB431542), followed by a cocktail of three small molecules (SU5402, CHIR99021 and DAPT) and subsequent treatment with ascorbic acid and cAMP, human embryonic (H9) and human induced pluripotent stem cells are effectively differentiated into putative Schwann cell precursors, using defined conditions. Results: Real time PCR demonstrates significant enrichment of PMP22, cadherin19, and myelin protein zero, and immunocytochemistry shows expression of S100B in the putative Schwann cell precursors. Additionally, we found that FACS for surface marker cd49d, an alpha4 integrin, allows for prospective isolation of this cell population following differentiation. Conclusion: Using a modified dual-SMAD inhibition protocol, we report the direct derivation and prospective isolation of Schwann cell precursors, in defined conditions.

W-3246

SELECTIVE ABLATION OF THE TUMOR AFTER NSCS TRANSPLANTATION BY CONTROLLING THE IMMUNE SUPPRESSION.

Go Itakura[1], Yoshiomi Kobayashi[1], Yuichiro Takahashi[1], Satoshi Nori[1], Soraya Nishimura[1], Morito Takano[1], Tsunehiko Konomi[1], Hiroki Iwai[1], Akio Iwanami[1], Yoshiaki Toyama[1], Masaya Nakamura[1], Hideyuki Okano[2]
[A]Orthopedic surgery, [B]Physiology, [1]Keio university, Tokyo, Japan

Previously, we reported functional recovery after transplantation of mouse as well as human induced pluripotent stem cell-derived neurospheres (iPSC-NS) into rodents spinal cord injury (SCI) models (Tsuji et al, PNAS 2010; Nori et al, PNAS 2011). Although iPSCs are useful for SCI treatment, tumorigenicity of transplanted cells should be resolved before clinical applications. In this study, we sought to determine the feasibility of the selective ablation of the grafted cells by controlling the immune suppression.
We used human glioblastoma cell line (U251) and neurospheres derived from a human iPSC line (253G1), which is already evaluated as an unsafe clone in our previous study. First, we transplanted U251 cells into the intact spinal cord of NOD/SCID mice as well as C57BL6J mice with immune suppressors. In vivo bioluminescent imaging (BLI) was used to evaluate the survival of transplanted cells chronologically. The graft survival rate was 100 % (n = 9/9) in the NOD/SCID mice group, 0 % (n = 4/4) in the C57/BL6J mice group without immune suppressor and 100 % (n = 11/11) in the C57/BL6J mice group with immune suppressors. After confirming the growth of the grafted cells in the C57/BL6J mice with immune suppressors, immune suppression was discontinued. Then the grafted cells were rejected immediately. Through the histological evaluation, we confirmed the ablation of the grafted cells.
Next, we transplanted the 253G1-derived neurosphere into the spinal cord of BALB/Ca mice with immune suppressors. The graft survival rate was 100% (n = 17/17). The BLI-photon counts of the grafted cells increased gradually 6 weeks after transplantation, followed by the deterioration of motor function. After discontinuing the immune suppression, we observed the immediate rejection of the grafted cells and motor functional recovery in all the mice (n = 6/6). Through the histological analysis, we also confirmed the ablation of the graft derived tumor.
Taking advantage of the immune rejection, controlling a graft survival could be a safety lock against iPSC-NS derived tumor in the cell therapy for SCI.

W-3247

ELECTRICALLY EXCITABLE SMALL NETWORKS OF NEURONS IN A HUMAN IPS 3-D SFEB SYSTEM.

Michael W. Nestor, Daniel Paull, Samson Jacob, Andrew A. Sproul, Abdulaziz Alsaffar, Brian A. Campos, Scott A. Noggle
The New York Stem Cell Foundation, New York, NY, United States

Three-dimensional aggregation cultures allow for complex development of differentiated human induced pluripotent stem cells. However, this approach is not easily amenable to live cell imaging and electrophysiological applications due to the thickness and the geometry of the tissue. Here, we present an improvement on the traditional aggregation method by combining the use of cell culture inserts with serum-free embryoid bodies (SFEBs). The use of this technique allows the structures to maintain their three-dimensional structure while thinning substantially. We demonstrate that this technique can be used for electrophysiological recording as well as live-cell calcium imaging combined with electrical stimulation, akin to organotypic slice preparations. This provides an important experimental tool that can be used to bridge 3-D structures with traditional monolayer approaches used in stem cell applications.

W-3248

SINGLE CELL CHARACTERIZATION OF MOTOR NEURONS DERIVED FROM HUMAN FIBROBLASTS AND PLURIPOTENT STEM CELLS

Sulagna Ghosh[1], Kevin Eggan[2], Steven McCarroll[1]
[1]Stanley Center for Psychiatric Research, The Broad Institute, Cambridge, MA, United States, [2]Dept. of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States

The recent advent of cellular reprogramming and iPS technology provides a powerful new means for modeling human diseases in a systematic, high-throughput and patient-specific manner. Despite the promise, effective modeling of complex neurological disorders has been hampered by our limited understanding of the inherent variability and heterogeneity of neuronal populations derived from pluripotent stem cells and patient fibroblasts. A method is needed to more rigorously classify in vitro derived neurons into discrete subtypes, both to diagnose the source

of cellular heterogeneity and to guide the development of standardized protocols for generating disease relevant cell subtypes. This study in particular aims to use single cell expression assays to better characterize the diversity and distribution of motor neuron subtypes in cultures generated via lineage specific reprogramming and transdifferentiation protocols. We anticipate that the results from this study will not only help model cell to cell variation in motor neuron cultures but also inform aspects of disease modeling that have previously been limited by cellular heterogeneity.

W-3251

DERIVATION AND ISOLATION OF NKX2.1-POSITIVE BASAL FOREBRAIN PROGENITORS FROM HUMAN EMBRYONIC STEM CELLS

Noelle D. Germain[1], Erin Banda[2], Michaela Tolman[2], Janice Naegele[2], **Laura B. Grabel**[2]
[1]Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, United States, [2]Biology, Wesleyan University, Middletown, CT, United States

Human embryonic stem cell (hESC)-derived ventral forebrain progenitors are ideal candidates for cell-based therapies that aim to replace dysfunctional or damaged cortical or hippocampal GABAergic interneurons. GABA-expressing interneurons are the major inhibitory cells of the cerebral cortex and hippocampus. These interneurons originate in the medial (MGE) and lateral (LGE) ganglionic eminences of the ventral forebrain during embryonic development and show reduced survival and function in a variety of neurological disorders, including temporal lobe epilepsy (TLE). While human ESCs (hESCs) are readily differentiated *in vitro* into dorsal telencephalic neural progenitors, standard protocols for generating ventral subtypes of telencephalic progenitors are less effective. We now report efficient derivation of MGE-like GABAergic progenitors using a modified monolayer neural differentiation protocol and an established hESC reporter line in which green fluorescent protein (GFP) is driven by the endogenous Nkx2.1 promoter. Consistent with sonic hedgehog (SHH)-dependent specification of NKX2.1-positive progenitors in the embryonic MGE, we show a dose-dependent increase in the generation of NKX2.1:GFP-positive progenitors following SHH treatment *in vitro*. Characterization of NKX2.1:GFP-positive cells confirms their identity as MGE-like neural progenitors, based on gene expression profiles and their ability to differentiate into GABAergic interneurons. We are also able to generate highly enriched populations of NKX2.1:GFP-positive progenitors by fluorescence activated cell sorting (FACS), and transplantation studies are underway to test the ability of these cells to differentiate and restore inhibitory activity in the mouse hippocampus.

W-3252

OPTIMAL TIME POINT OF NEURAL STEM CELL TRANSPLANTATION THERAPY IS AT THE SUB-ACUTE PHASE OF SCI IN NON HUMAN PRIMATE

Soraya Nishimura[1], Kenji Yoshida[6], Hiroki Iwai[1], Yoshiomi Kobayashi[1], Shinsuke Shibata[2], Hayao Ebise[6], Ikuko Koya[2], Takashi Sasaki[3], Atsushi Shimizu[4], Jun Kudoh[5], Yoshiaki Toyama[1], Masaya Nakamura[1], Hideyuki Okano[2]
[A]Dept of Orthopaedics surgery, [B]Dept of Physiology, [C]Center for Integrated Medical Research, [D]Dept of Molecular Biology, [E]Laboratory of Gene Medicine, [1]Keio Univ. Sch Med, Tokyo, Japan, [2]Genomic Science Laboratories, Dainippon Sumitomo Pharma Co., Ltd.,, Osaka, Japan

INTRODUCTION: The microenvironment of injured spinal cord significantly affects the survival, the differentiation phenotype and the distribution of neural stem cells (NSCs) transplanted into the spinal cord. Analyses of microenvironmental changes of injured spinal cord, which are critical to determine a therapeutic time-window of NSCs transplantation for spinal cord injury (SCI), was previously reported in rodent SCI models, but not in a non-human primate SCI model. Here, we examined the global gene expression analyses to investigate microenvironment of injured spinal cord in adult common marmosets (Callithrix jacchus) to determine an optimal time-window of NSCs transplantation therapy in a primate SCI model.

METHODS: Contusive SCI was induced at C5 level in adult female common marmosets using a modified NYU impactor as reported previously. The injured spinal cord samples were harvested from each experimental animal at 1, 2, 4 and 6 WPI. Transcriptome analyses, such as microarray and next generation sequencing, were performed using these samples. The control animals received only laminectomy without SCI. Histological analyses including immunostaining for GFAP, Iba-1 and chondroitin sulfate proteoglycans (CSPG) of injured spinal cord were also performed at 1, 2 and 6 WPI.
RESULTS: Transcriptome analyses revealed that the expressions of genes associated with inflammatory cytokines, reactive oxygen and T cell activation were significantly up-regulated at 1 WPI and decreased at 2 WPI and thereafter. In contrast, the expressions of genes about synaptic transmission and neurotransmitter secretion were down-regulated at 1 WPI and elevated at 2 WPI and thereafter. Next, we focused on each indivisual gene expression. The expression of transforming growth factor (TGF) β, which was known as anti-inflammatory cytokine was drastically increased at 1 WPI and then decreased at 2 WPI followed by a gradual decrease until 6 WPI. In contrast, the expressions of most pro-inflammatory cytokines were already declined at 1 WPI. The markers of macrophages such as CD14 and CD68 were dramatically up-regulated at 1 WPI and then decreased rapidly to the control level at 2 WPI, whereas the expression of CSPGs was increased at 2 WPI and thereafter. Histological analysis revealed that more macrophages or microglial cells infiltrated around the lesion epicenter at 1 WPI than at the other time-points. Immunohistology for GFAP and CSPG showed that the glial scar formation and the CSPG accumulation were more prominent at 6 WPI compared with that at 1 and 2 WPI.
CONCLUSIONS: Taken together, the acute inflammatory response after SCI was diminished at 2 WPI, however the glial scar formation and the CSPG accumulation were already irreversible at 6 WPI. These findings suggested that the optimal time-window of NSCs transplantation might be around 2 to 4 WPI at the sub-acute phase of SCI in non-human primates.

W-3253

IMPROVEMENT OF MOTOR AND SENSORY FUNCTIONS IN A RODENT MODEL OF CONTUSED SPINAL CORD INJURY FOLLOWING TRANSPLANTATION OF BDNF-OVEREXPRESSING HUMAN NEURAL STEM CELLS (F3.BDNF)

Da-Jeong Chang[1], Hwi-Young Cho[2], Dong Ah Shin[3], Seyoung Hwang[1], Nayeon Lee[1], Iksoo Jeon[1], Chunggab Choi[1], Seung-Hun Oh[1], Hyun Sook Kim[1], Hyunseung Lee[4], Kwan Soo Hong[4], Young Wook Yoon[2], **Jihwan Song**[1]
[1]CHA Stem Cell Institute, Department of Biomedical Science, CHA University, Seoul, Korea, Republic of, [2]Department of Physiology and Neuroscience Research Institute, Korea University College of Medicine, Seoul, Korea, Republic of, [3]Department of Neurosurgery, Yonsei University College of Medicine, Seoul, Korea, Republic of, [4]Division of Magnetic Resonance, Korea Basic Science Institute, Ochang, Korea, Republic of

Spinal cord injury (SCI) represents the major form of paralysis and disabilities, but its therapeutic option is currently very limited or none. Numerous efforts have been made to cure SCI using stem cells, which gave rise to some promising results in animal experiments. However, since the halt of human embryonic stem cell-based clinical trial by Geron, further efforts to develop more efficient stem cell-based transplantation strategies in SCI became less active or minimized. In this study, we evaluated the therapeutic potential of human neural stem cells overexpressing BDNF (F3.BDNF) in a rodent model of contused spinal cord injury. BDNF (brain-derived neurotrophic factor) is known to play important roles in neuronal survival, neuroprotection, axonal regeneration, etc. F3.BDNF cells ($1x10^6$ cells in 10 μl) were delivered to the injury site intrathecally at 7 days after contusion injury at T11 level, and the transplantation effects were monitored up to 10 weeks using animal MRI and behavioral analyses. 4.7T MRI analysis indicated that Feridex-labeled F3.BDNF cells were mainly populated in the injury site, of which size was shown to be significantly reduced, compared with the sham control group. Histological analysis further revealed that the volume of lesion cavity was significantly reduced, whereas the extent of myelination was greatly increased in the F3.BDNF cell-transplanted group. Importantly, the transplanted animals exhibited improved behavioral recovery, judged by BBB and CBS tests. We also observed that transplanted F3.BDNF cells gave rise to significant sensory recovery, according to von Frey pain test. Immunocytochemical staining indicated that the transplanted cells were differentiated into both neural and glial lineages. Retrograde labeling using Fluoro-Gold demonstrated that the transplanted cells

were engrafted into the host tissue. We also found that the transplanted animals exhibited reduced numbers of Iba1, iNOS and GFAP-positive cells, suggesting that they can also modulate inflammation and gliosis after SCI. Taken together, these results indicate that human neural stem cells overexpressing BDNF can contribute to the functional improvement in a rodent model of SCI significantly, providing a new therapeutic possibility to treat SCI patients in the future.
This study was supported by a grant of the Korea Healthcare technology R&D project, Ministry for Health, Welfare & Family Affairs (A111016). We are grateful to the MRI facility in the Division of Magnetic Resonance, Korea Basic Science Institute, Ochang, Korea.

W-3254

DEFINITIVE NEURAL STEM CELLS CLONALLY GENERATED FROM PLURIPOTENT STEM CELLS PROMOTE RECOVERY FOLLOWING SPINAL CORD INJURY

Ryan Salewski, Robert A. Mitchel, Michael G. Fehlings
Institute of Medical Science, University of Toronto, Toronto, ON, Canada

Despite advances in medical and surgical care, current clinical therapies for spinal cord injury (SCI) have limited effectiveness. Axonal demyelination is a key pathogenic mechanism following SCI that leads to an interruption of signal transmission and contributes to axonal degeneration. Cell-based therapies using neural stem cells (NSC) animal models of SCI have shown positive outcomes. Pluripotent cell sources such as embryonic stem cell (ESC) and induced pluripotent stem cell (IPSC) provide a limitless supply of therapeutic cells, the later allowing for patient-specific treatments. NSCs derived using embryoid bodies (EB) from ESC/IPSC in transplant and have been shown tumorigenic potential. The default pathway of neutralization may be used as an alternative method to generate safer and more clinically relevant NSCs without an EB intermediate for use in SCI cell-based therapy.
Using the default pathway we generate clonally derived definitive NSC (dNSCs) from ESC and IPSC sources. These cells are transplanted in a mouse thoracic spinal cord injury model. dNSC-transplanted mice were compared to control mice using behavioral measures (BMS, gait analysis) and histopathological analysis of tissue as well as the role of remyelination in injury recovery was investigated using transmission electron microscopy.
The SCI group that received dNSC transplantation showed significant improved in locomoter function compared to control injury in BMS evaluation. The gait analysis showed improved swing speed, stride length and coordinated stepping compared to controls. Furthermore the cell treatment group had a greater total area of spinal cord, grey matter and a trend towards increased white matter. Immunohistological assessments 8 weeks post injury showed that dNSCs differentiated to primarily to OLIG-2 positive oligodendrocytes. These cells were shown to expressed myelin basic protein, associated with axon and displayed proper compact, multilayer myelination.
This study suggests that dNSCs clonally derived from pluripotent cell using the default pathway of neuralization improves motor function following SCI, while remaining safe and clinically relevant.

W-3255

A MICRORNA PROFILE OF REDUCED MIR-34B/C AND INCREASED MIR-592 IN ADULT EPILEPTIC PATIENT-DERIVED BRAIN CELLS REVEALS POTENT DISEASE BIOMARKER AND SCREENING TOOL FOR TRANSPLANTABLE STEM CELLS

Naoki Tajiri[1], Hiroto Ishikawa[1], Kazutaka Shinozuka[1], Travis Dailey[1], Yuji Kaneko[1], Teresita J. Malapira[1], Carmelina Gemma[2], Fernando Vale[1], Cesar V. Borlongan[1]
[1]Neurosurgery & Brain Repair, Univ of South Florida Morsani College of Medicine, Tampa, FL, United States, [2]Anesthesiology & Pain Medicine, Univ of Washington, Seattle, WA, United States

Over three million Americans suffer from some form of epilepsy, with mesial temporal lobe epilepsy (TLE) being the most common, arising from the temporal lobe structures of amygdala, hippocampus and parahippocampal gyrus. While many antiepileptic drugs are available, temporal lobectomy is the definitive treatment for intractable TLE. The present study sought to further understand the pathophysiology of epilepsy in an effort to develop more ef-

fective treatments for this disorder. Under USF IRB approval, tissues from multiple brain regions were obtained from consenting TLE patients undergoing hippocampal resection. Specimens were processed for microRNA and RT-qPCR analysis, while alternate tissues were grown in culture for immunohistochemical assays. Approximately 800 microRNAs were examined. Results revealed that miR-34b and miR-34c were significantly upregulated in hippocampus and amygdala compared to the neocortex, with miR-34b/c expression highest in the amygdala. In contrast, levels of miR-592 were significantly downregulated in the hippocampus and amygdala compared to the neocortex. Sister brain tissues of profiled microRNA samples were then processed for RT-qPCR focusing on the identified novel microRNAs. RT-qPCR data generally support microRNA data. Notably levels of miR-34b/c were significantly upregulated, while levels of miR-592 were downregulated in the hippocampus and amygdala compared to neocortex. In tandem, immunocytochemical staining against stem cell markers and cell survival/death provided insights into the function of these miRs, in that distinct cell growth patterns were recognized from stem cells harvested from brain-specific regions with cell proliferation and differentiation reduced in the hippocampus and amygdala compared to the neocortex of TLE patients. Intra-amygdalar transplants of stem cells derived from the neocortex (i.e., reduced miR-34b/c but elevated miR-592 expression) of epileptic patients not only survived in the amygdala and migrated to the hippocampal lesions, but also reduced hippocampal cell loss in rats subjected to kainic acid-induced epilepsy. Parallel in vitro studies revealed that the administration of the supernatant from cultured human epileptic neocortical stem cells significantly reduced the kainic acid-induced cell death in primary human hippocampal cells compared to control treatments, suggesting that the rescue of the epileptic hippocampus by the transplanted stem cells likely involves a trophic factor mechanism. This study elucidates the key role of microRNAs in the disease pathology of epilepsy and their utility as a stem cell optimization tool for identifying efficacious stem cells for transplantation therapy. A better understanding of the role of microRNAs in the disease pathology of TLE may lead to novel (i.e., stem cell-based) biomarkers and treatments for epilepsy and relevant disorders.

Thursday Posters

Education and Outreach

T-1001

LIFEMAP^{TM} DISCOVERY- THE STEM CELLS, EMBRYONIC DEVELOPMENT AND REGENERATIVE MEDICINE RESEARCH PORTAL

Ronit Shtrichman, Ron Edgar, Yaron Mazor, Idit Livnat, Ariel Rinon, Shani Ben Ari, Ella Buzhor, **Jacob Blumenthal**, Netta Shraga, Yoel Bogoch,, David Warshawsky
LifeMap Sciences Ltd., Tel Aviv, Israel

Understanding how cells differentiate during embryonic development is invaluable for the derivation of functional cells from stem cells *in vitro*. The LifeMap Discovery database has been created to bridge these two research areas, and bring knowledge from the *in vivo* into the *in vitro*. Knowing the genes expressed in developing cells and the signaling that derives their differentiation is essential information for identification and classification of stem cells, and suggestive mechanisms to help develop differentiation protocols and therapeutic products.
LifeMap Discovery, available free of charge to all academics at http://discovery.lifemapsc.com, traces the cellular differentiation that occurs during mammalian development as well as information of stem cells differentiation. The database is based on systematic assimilation of scientific data describing developmental paths to their fates. The database includes cells and anatomy development, supplemented with qualitative gene expression, signaling pathways, *in situ* hybridizations and high throughout experimental data, related diseases, images and relevant references.
The database is divided into the following parts:
1.
In vivo development - Cell lineages developed in the mammalian body.
2.
Stem cell differentiation - cultured cells and differentiation protocols.

3.
Gene expression and Signaling - how and where genes are expressed, what signals contribute to differentiation.
4.
Regenerative Medicine - development of stem cells into therapeutic products.
These different parts are connected and interlaced by computational and hand curated methods, most noteworthy, the *in vivo* entities are linked to their closest *in vitro* entities, based on gene expression analysis.
The value provided by LifeMap Discovery originates from the combined power of this data, which enables identifying, predicting and indicating possible differentiation paths and future regenerative medicine applications. LifeMap Discovery integrates symbiotically with the more elemental database GeneCards where rich information is available at the gene level, and MalaCards, that provides human disease information.

T-1002

STEMBOOK, A RESOURCE FOR STEM CELL RESEARCHERS AND EXTENSIBLE PLATFORM FOR BIOMEDICAL WEB COMMUNITIES

Lisa Girard*[1], Sudeshna (Co-presenter) Das*[2], Stephane Corlosquet[3], Paolo Ciccarese[2], Timothy W. Clark*[2], Brock (co-senior author) Reeve*[1]

[1]Harvard Stem Cell Institute, Cambridge, MA, United States, [2]MIND Informatics, Massachussetts General Hospital, Harvard Medical School, Boston, MA, United States, [3]MIND Informatics, Massachussetts General Hospital, Boston, MA, United States

StemBook (www.stembook.org), is an original, open-access, peer-reviewed “book” with chapters covering topics in stem cell biology, commentaries, protocols, journal clubs, news, and forums. Written by top researchers in the field worldwide and geared toward stem cell and non-specialist researchers, but also appropriate within an undergraduate and graduate developmental biology curriculum, StemBook is divided into sections that span the basic to the translational. It represents a one-of-a-kind background resource with an online infrastructure equipped to continue evolving along with the field it covers. There are currently over 80 commissioned chapters and over 60 published chapters in StemBook. Each chapter represents a citable reference and is indexed in PubMed by the National Library of Medicine and appears on the NCBI Bookshelf. Since StemBook’s launch in September 2008, readership continues to grow dramatically and averages approximately 10,00 unique visitors each month.
The Scientific Collaboration Framework (SCF), co-developed by the MIND Informatics team at Massachusetts General Hospital, and the Harvard Stem Cell Institute, is a reusable platform for advanced structured digital collaboration. The first instance of this framework was used to create StemBook and several other web communities are now participating in the SCF ecosystem. SCF makes heterogeneous resources available to the collaborating scientist and captures the semantics of the relationship among the resources. The technology allows the wealth of StemBook content to be processed and mined.
Newly-enabled features include online journal clubs, news feeds and editor-moderated discussion forums. We are also creating user interfaces to support text mining and annotation of articles with stem cell vocabulary and resources. We are using a state of the art annotation tool, DOMEO (www.annotationframework.org) to markup StemBook articles. The proteins, biological processes, cell types/lines and hypotheses/claims within these articles are annotated with controlled vocabulary/ontology terms using the DOMEO tool. Such an annotation process provides an added service to the readers, whereby they can search for a term of interest and can retrieve the articles as well as the relevant context within the article. The application of newly-available technologies for semantics-based linking of biomedical web communities enables a new level of agile trans-disciplinary and translational collaboration in applications of

stem cell biology and regenerative medicine and will enable faster progress towards new clinical applications of stem cells.
Incorporating these features in the context of a top quality collection of original content geared directly at the stem cell research community creates a powerful means of reaching this reader base.
In collaboration with the NIH Center for Regenerative Medicine, StemBook has recently launched a methods and protocols section that provides readers with high quality, validated protocols for a wide range of stem cell research techniques and is overseen by StemBook but involves the coordination of over 10 major stem cell core facilities around the country. This section is transitioning to a forms-based approach with WYSIWYG capabilities with which users will be able to directly submit protocols, which will then be validated by the core facilities and potentially published as a vetted protocol.

T-1003

ETHICS OF TRANSLATIONAL STEM CELL RESEARCH: WHAT VALUE JUSTIFIES FIRST-IN-HUMAN TRIALS?

Annelien L. Bredenoord, Johannes JM Van Delden
Medical Ethics, University Medical Center Utrecht, Utrecht, Netherlands

Pluripotent stem cell (PSC) research has great potential for regenerative medicine, but is at the same time accompanied by a fierce ethical and political debate. This debate, however, has almost exclusively focused on the contested moral status of the human embryo. There is however a set of ethical issues relevant to both human embryonic stem cell and induced pluripotent stem cell research that are not related to the moral status of the embryo: the ethical issues associated with moving PSCs from bench to bedside. The first PSC trials have been initiated and more are under way. In this paper we will examine the ethical specifics of early-phase PSC trials.
First-in-human trials are ethically challenging by nature, particularly because the needed evidence to reliably predict risk and benefit (testing in humans) is missing. Whereas many innovative interventions raise ethical issues, PSCs trials are a new cutting edge where many ingredients come together including the unique risks and characteristics of PSCs, the inherent uncertainty of first-in-human studies, the sensitivity of the preclinical research, the relative unreliability of animal models, the potential vulnerability of research participants, the tremendous scientific and commercial stakes and the high public and political attention. In the initial applications of a first-in-human PSC trial it is likely that the risks and uncertainties are higher than the direct-benefits for the research participants. As such a trial cannot be deemed therapeutic, the question becomes whether early-phase PSC trials can be justified otherwise when potential risks and uncertainties outweigh direct-benefits to research participants. A possible answer to this ethical dilemma is that such trials could still be morally acceptable when the *value* of the study is sufficient to justify the risks, i.e. there are sufficient potential benefits to science and society (instead of direct-benefits to participants). This, however, raises some further questions that are relatively unaddressed yet.
The concept of value is brought to bear in all influential codes of research ethics but is scantly discussed and elucidated. What exactly entails value in clinical research, and what kind of value is a trial expected to generate? If this is for example knowledge, then one could wonder how compatible this is with the Declaration of Helsinki that states that “the well-being of the individual research subject must take precedence over all other interests” (article 6). A strict interpretation of the Declaration of Helsinki may therefore imply that many if not most first-in-human studies are unjustifiable.
If the ethical permissibility of first-in-human PSC trials can be justified with an appeal to value, then this sets several additional criteria, for example regarding outcome measures, scientific design, value maximization, risk minimization, knowledge utilization, participant selection and informed consent.

T-1004

CURRENT JAPANESE GUIDELINES ON ENSURING QUALITY AND SAFETY OF PRODUCTS DERIVED FROM PROCESSING OF HUMAN STEM CELLS

Takao Hayakawa[1], Takashi Aoi[2], Akihiro Umezawa[3], Keiya Ozawa[4], Yoji Sato[5], Yoshiki Sawa[6], Akifumi Matsuyama[7], Shinya Yamanaka[2], Masayuki Yamato[8]

[1]Pharmaceutical Research and Technology Institute, Kinki University, Osaka, Japan, [2]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [3]Department of Reproductive Biology, National Research Institute for Child Health and Development, Tokyo, Japan, [4]Division of Hematology, Department of Medicine, Jichi Medical University, Tochigi, Japan, [5]Division of Cellular and Gene Therapy Products, National Institute of Health Sciences, Tokyo, Japan, [6]Department of Surgery, Osaka University Graduate School of Medicine, Osaka, Japan, [7]Division of Regenerative Medicine Development Assistance, Foundation for Biomedical Research and Innovation, Hyogo, Japan, [8]Advanced Biomedical Science Center, Tokyo Women's Medical University, Tokyo, Japan

Regenerative medicine using relevant products
derived from various human stem cells are being keenly anticipated in Japan due to limited resources of human organs and tissues transplantation and difficulty of treatments to cure various severe diseases. The more advances in stem cell science, the more people expect the effective and efficient translation into regenerative medicine. Identifying points to consider for ensuring quality, safety and efficacy of products derived from the various types of stem cells is crucial through product development for their rapid application in patients. Technical requirements for manufacturing and marketing authorization of cells/tissue-based products under the Japanese Pharmaceutical Affairs Law, are described in the Notification No. 1314 of Secretary-General of Pharmaceutical and Food Safety Bureau (PFSB), Ministry of Health, Labour and Welfare (MHLW), dated December 26, 2000. The 2006/2007 Scientific Research Group of the MHLW looked into preparing a revised version of "Guideline on Ensuring Quality and Safety of Products Derived from Processing of Human Cells/Tissue", which is Appendix 2 in the PFSB/MHLW 1314, in response to requests that Japan should push forward with appropriate regulations for regenerative medicine through the revision of assessment criteria that reflects rapidly developing science and technology, ethical viewpoints, and international trends. This revision, splitting the original guideline up into 2 different guidelines in order to make clearer the specific technical requirements for products derived from autologous cells and allogenic cells, led to the publication of the "Guideline on Ensuring Quality and Safety of Products Derived from Processing of Human Autologous Cells/Tissue" (PFSB/MHLW Notification 0208003) in February 2008, and the "Guideline on Ensuring Quality and Safety of Products Derived from Processing of Human Allogenic Cells/Tissue" (PFSB/MHLW Notification 0912006) in September 2008, respectively. However, further studies became necessary to identify specific issues related to products derived from processing of somatic stem cells, embryonic stem cells or induced pluripotent stem cells (iPSCs), all of which have been attracting a lot of attention in recent years. At this point, the MHLW decided to form in fiscal year 2008. a panel of experts (the authors of this presentation) for drafting guidelines on ensuring the quality and safety of products derived from processing of various human stem cells. At the presentation, we address the current guidelines concerning the basic technical requirements for ensuring the quality and safety of products derived from processing of human stem cells, which the MHLW released on September 7, 2012. The guidelines reflect the results of the study on the current situation

and future outlook, from a scientific and technological perspective, of the manufacture and clinical application of human stem cell-based products.

T-1005

REGULATION OF CELL AND TISSUE THERAPY - A WAY FORWARD FOR UNPROVEN INTERVENTIONS

Peter ONeill, Chih W. Teng
Management, Monash University, Melbourne, Australia

On 23th of July 2012, a district court in Washington DC ruled in favour of FDA seeking an injunction against Regenerative Sciences Inc., a company providing autologous stem cell therapy for orthopaedic injuries. The ruling of the court has given legitimacy to the FDA's push to regulate autologous therapies in the US. This may mean that autologous therapies, which are classified as excluded goods, will have to conform to the same review process as drugs, and be regulated in much the same way. This occurs against the background of the increasing number of private stem cell clinics and stem cell tourism, which largely go unregulated. This phenomenon occurs not only in the US, but also in countries such as the UK and Australia. Whether the decision by the district court works towards the progress of the field is spilt between two views.

Members of the scientific and bioethics community who favour the decision have cited the following prominent arguments. Firstly, robust methods must be applied to all therapies to ensure clinical results can be validated through a certified test/trial regime. Secondly, clear governance structures need to be in place to protect the patient from misleading claims.

The counterpoint adopted by professional and practitioner groups argues that the FDA has encroached into the practice of medicine. By adopting a legalistic approach the FDA has breached the sacrosanct line that separates the regulation of medical practice from the regulation of medical products. Furthermore, commercial entities and some patient advocacy groups contended that translation and commercialization of autologous cell therapies would be delayed if the current lengthy drug review process were instigated.

We thus contend that:

1. Funding providers such as insurance agencies and medicare heavily influence the rate of an innovations adoption into routine practice. Such entities provide financial assistance via subsidies and reimbursements that help offset the costs to patients. Without regulator approval or endorsement by relevant authorities, it is expected that the rate of adoption will slow tremendously.
2. Practitioner led commercialisation is heavily influenced by the ability to leverage on technology platforms that help deliver positive patient outcomes. Without access to enabling technologies that help deliver therapies closer to points of care, it is expected that the rate of adoption will decline.

Thus in terms of maximising the greatest good from these investments, it is paramount that attempts are made to reconcile conflict between rate of the commercialisation process and the rate of adoption.

Using Diffusion Theory as a theoretical lens for our case study, we collected information through interviews with key representatives of the international regenerative medicine industry.

Our findings suggest that to satisfy ISSCR Guidelines for the Clinical Translation of Stem Cells (2008:15-16) Recommendation 34, and the Australian TGA Excluded Goods Order Number 1 2011, a regulatory framework should encompass the following:

1.
The licencing of clinician-scientists (at practice level) to perform (excluded/autologous) stem-cell procedures
2.
The establishment of a controlled register of procedures that can be performed
3.
All patient data must be maintained on their personal-e-health record (and accessible by researchers)
4.
Limitations to the extraction of cell types at practice level
5.
Limitations of cellular manipulation ex vivo at practice level

Society Issues

T-1006

LIFELINES: STEM CELL RESEARCH IN A GLOBALIZED WORLD

Brooke M. Ellison
School of Health Technology and Management, Stony Brook University, Stony Brook, NY, United States

Matters of science traditionally have been looked at from two sociological perspectives. The Sociology of Scientific Knowledge (SSK) argues that science is similar to other socially constructed phenomena, and is a product of context. From this perspective, science and culture have an inextricable relationship. World Polity Theory (WPT), on the other hand, makes the argument that science is a rationalistic entity that exists outside of cultural forces. However, as science becomes increasingly complex and multidimensional, these two theories leave room for additional analysis. Bourdieu's Field Theory brings to light how interactions between social actors make certain social realities possible, and create the environments for the development of policy matters. This theoretical approach is a necessary addition to better understand science as it is codified in policy. The scientific field of embryonic stem cell research is an ideal site to apply these theoretical frameworks.
Stem cell research is a matter of biology that incorporates complex epistemic questions like the status of life, respect for human dignity, and the value of science as a source of economic production. As a result, the policy surrounding human embryonic stem cell research takes on social contestation not directed to other areas of scientific investigation even within the biomedical sector. The globalized nature of stem cell research is seen in organizations like the International Society for Stem Cell Research (ISSCR). The culturally-determined nature of stem cell research is seen in the diversity of local concerns that it highlights: concerns that incorporate ethical, epistemic, and economic questions. Both of these competing ideas come into play as policymaking entities and powerful social actors negotiate ways to address the stem cell question.
This analysis investigates stem cell research legislation in four contexts: the US, UK, Germany, and China. Each of these cases presents a set of considerations, which changes the landscape of the political field in which stem cell research has evolved. In these cases, the impact of four social actors has been addressed: the Catholic Church, scientific community, pharmaceutical industry, and patient advocacy community. How these actors operate in each context is dependent on a confluence of social forces. The US' stem cell policymaking has been defined by a battle for epistemic authority on issues of life. The UK case illustrates the role of expertise as authority in democratic policymaking. The German case illustrates the ways in which collective memory of iconic events operates through social actors. And, China represents a control case, indicating how policy can evolve in circumstances where the political field is limited. Taken together, these cases illustrate how Sociology of Scientific Knowledge, World Polity Theory, and Field Theory can work together to explain complex modern science.
This analysis demonstrates the need for a more synthetic approach among analytical frameworks. In the US and Germany, there is a stem cell policy that has been characterized by some degree of ethical concern. Alternatively, in the UK and China, there is a stem cell policy that has been characterized by a degree of liberality. The result is four cases, two with a restrictive stem cell policy and two with a progressive stem cell policy, yet each with a set of dynamics that shapes the social field and influence how social actors operate within it.

T-1007

EMERGING PRIVACY CHALLENGES IN CELL THERAPY RESEARCH: THE CANADIAN CONTEXT

Sarah Burningham
Faculty of Law, University of Alberta, Edmonton, AB, Canada

Innovations in cell therapy research have the potential to revolutionize health care. However, the need for robust privacy protection of genetic information is particularly important in the context of cell therapy research, given policies, introduced to address pragmatic and logistical concerns associated with consent and withdrawal of consent, which have limited the rights of individuals to control and withdraw their tissue from research. For example,

most biobanks now use broad consent models and some policies and regulations limit the right of individuals to withdraw consent to tissue research to a time before a cell line is created.
Cell therapy research presents unique challenges for privacy regulation. While cell therapy research generally involves funding and research collaboration by multiple private and public actors, privacy regulation is traditionally divided on public-private lines, making application of privacy statutes to a given project challenging. Additionally, several recent studies have demonstrated that de-identified genetic information can be re-identified by combining this information with other identifying information available in publicly-accessible databases, challenging the notion that de-identification is a sufficient guarantee of privacy in this context. However, privacy legislation in Canada does not apply to de-identified information, meaning it falls outside the scope of legislative protection.
My objective is to provide an overview of privacy regulation of cell therapy research in Canada. I will identify challenges associated with privacy regulation and highlight gaps in the current legislative and judicial frameworks that apply to this research. In particular, I will argue that reform is needed to address the risks of re-identification respecting de-identified genetic information, as such information is currently excluded from the scope of privacy regulation.

T-1008

A COLLABORATIVE ROLE FOR PATIENT ADVOCATES IN DEVELOPING REGULATORY SCIENCE FOR GOVERNMENT APPROVAL OF STEM CELL-BASED AND REGENERATIVE MEDICINES

Alan L. Jakimo[1], Bernard Siegel[2]
[1]Sidley Austin LLP, New York, NY, United States, [2]Genetics Policy Institute, Wellington, FL, United States

Stem cell science and regenerative medicine have benefited during the past decade from the efforts of patient advocates. Continuation of these efforts from patient advocates will be a key to the success of the increasing number of stem cell-based and regenerative medicines that undergo clinical translation and reach the stage of consideration by government regulators charged with approving market authorization applications submitted by the sponsors of these medicines. This raises the question as to whether patient advocates are sufficiently schooled in the language and dynamics of the regulatory approval process so that they can make a meaningful and thoughtful contribution to that process. At the same time, as a general matter, government regulators responsible for approving medical products are focusing their efforts on improving existing regulatory science tools and developing new ones. (FDA defines regulatory science as “the science needed to assess and evaluate a product’s safety, effectiveness, quality, and performance” and suggests that it “can be a critical link between cutting-edge discoveries and real-world diagnostics, treatments, and cures.”) Here we propose that patient advocates can play an important role in the development of regulatory science for medical products arising from SCS&RM.
First, we review how patient advocates have historically participated in the regulatory approval process, particularly with respect to medical products related to SCS&RM. Second, we review current efforts by government regulators, such as FDA, to improve regulatory science in general. Third, we suggest specific elements of regulatory science that need to be improved or freshly developed for SCS&RM-related medical products. Fourth, we hypothesize how patient advocates can participate in this process of improving and developing regulatory science for SCS&RM-related medical products.
We test our hypothesis by reference to a specific instance of the regulatory approval process for an SCS&RM-related medical product and the ongoing efforts of FDA in the field of regulatory science. We observe here the importance of collaboration between and among patient advocates and other stakeholders.
We conclude by suggesting a series of symposia in venues around the globe for training patient advocates in the intersection of regulatory science and the government approval process as it relates to SCS&RM medical products and in building relevant collaborations with other stakeholders. These symposia will require the participation of scientists and physicians.

Cancer Cells

T-1011

INDUCED TUMOR GENERATING CELLS (ITGC) FOR A WIDE EVOLUTIONARY RANGE OF SPECIES

Ricardo A. Rossello[1], Ute Hochgeschwender[2], Rui Dai[2], Erich D. Jarvis[3]

[1]Biochemistry, University of Puerto Rico Medical Sciences Campus / University of Puerto Rico Comprehensive Cancer Center, San Juan, PR, United States, [2]Neurobiology, Duke University, Durham, NC, United States, [3]Neurobiology, Duke University / Howard Hughes Medical Institute, Durham, NC, United States

Cells are fundamental units of life, but little is known about evolution of cell states. Induced pluripotent stem cells (iPSCs) are once differentiated cells that have been re-programmed to an embryonic stem cell (ESC) state, providing a powerful platform for biology and medicine. Previously, our group published findings that showed iPSC-like cells can be induced in vertebrate and invertebrate model organisms that span 550 million years from a common ancestor: in mammals, birds, fish, and fly. Parallel to this effort we noticed a large number of transformed colonies that were being formed. These cells were not stem cells, but, in some cases, had transformed characteristics. Here, we examine these cells for mouse, chicken, and quail, and noticed they had a variety of features. First, some of the cells expressed a high level of telomerase activity while others had none. These cells were separated into two distinct subsets. The first group had significantly high level of proliferative capacity, while the second group did not. Secondly, they all expressed OCT-4, TERT, SSEA1, KLF-5, and ERAS.
Because they expressed pluripotent markers, additional to Oct-4, Sox-2, KLF-4, and C-myc, a pluripotent state seemed to be present. Surprisingly the slow proliferating group contained a larger set of pluripotent markers than did the fast, self renewing cells. These cells were injected into mice testes to assess teratoma formation. However, in both cases, the tumor that ensued was unstructured, suggesting carcinogenicity. In addition, to reject the possibility of these cells being induced stem cells, we injected them into embryos. In all cases (n=10, per species), embryogenesis was halted.
To further characterize these cells _____

T-1012

Targeting Cancer Stem Cells by “Signal-Smart” Oncolytic Viruses

Kaoru Terai Terai, Zhengian Liu, Emma Borrego-Diaz, Ayten Gadashova, Judith L. Larson, Tuba Esfandyari, **Faris Farassati**

Medicine, The University of Kansas-School of Medicine, Kansas, KS, United States

Cancer stem cells (CSCs) comprise a novel concept in molecular oncology. According to this concept, in resemblance to normal tissues, tumors contain a fraction of cells in charge of maintaining the functional and structural integrity of the tumor while generating different types of cells forming the malignant mass. These cells are referred to as CSCs and have been shown to be highly resistant to conventional chemo and radiotherapy. Although different studies have proven the validity of the concept of CSCs, the translational correlation between this concept and cancer therapy remains to be explored. In other words, if targeting cancer stem cells can result in a robust tumor regression remains as an attractive topic for investigation. In this regards, we have developed a novel version of oncolytic herpes viruses with the capability to target CSCs on cell-specific basis.
Oncolytic viruses are a promising class of replication competent viruses that in many cases, such as herpes model, have passed phase I clinical trials. These viruses replicate conditionally in the cancer cells leading to their destruction. Oncolytic virus therapy is based on the ability of viruses to effectively infect and kill tumor cells with minimal or no effects on normal tissues. While some viruses seem to have a natural preference for tumor cells, most viruses require the modification of their tropism to specifically enter and/or replicate in cancer cells. To this end, we have developed the first mutated version of herpes simplex virus-1(HSV-1) which is capable of replication in CSCs in a specific manner. CD133 is one of the most important markers for cancer stem cells involved in the biology of a number of human cancers. The virus developed in this work, referred to as Signal-Smart 2 or SS2 virus, shows specificity against CD133+ cells in three different models (liver cancer, colorectal cancer and melanoma) resulting in the loss of viability and invasiveness of these cells and eventual demise of the whole tumor cell population. The SS2 virus also showed a robust inhibitory activity against the growth of related tumor cells *in-vivo*. Additionally,

pre-established tumors regressed significantly once treated with this virus. The results of this study, not only introduce SS2 as a novel agent for targeting CSCs, but also establish CSCs in general (and CD133+ cells in specific) as a relevant target for future development of anti-cancer therapeutics. On the other hand, the concept of CSCs, as the main driving force behind the integrity of the tumor is further established by our studies.

T-1013

HETEROGENEITY OF SINGLE CELL GENE EXPRESSION ACROSS PHENOTYPICALLY DISTINCT POPULATIONS OF SINGLE CELLS AS MEASURED BY RNASEQ.

Anne Leyrat, Joseph Shuga, Nianzhen Li, Brian Fowler, Peilin Chen, Jing Wang, Suzanne Weaver, Marc Unger, Jay A. West
Research and Development, Fluidigm, South San Francisco, CA, United States

HETEROGENEITY OF SINGLE CELL GENE EXPRESSION ACROSS PHENOTYPICALLY DISTINCT POPULATIONS OF SINGLE CELLS AS MEASURED BY RNASEQ.
J.F. Shuga, A. Leyrat, N. Li, B. Fowler, P. Chen, X. Wang, J. Wang, S. Weaver, M. Unger1 & J. A. West1,
1New Technologies, Fluidigm Corporation, South San Francisco, CA
Multi-cellular populations are fundamentally driven by the collective properties of individual cells. However, our understanding of gene expression dynamics derived from cultures or tissues are based on measurements made from the entire population. A growing body of data collected from individual cells has challenged these basic assumptions. The data suggest that properties driving lineage, development and disease emerge from the transcriptional heterogeneity and signaling architectures of distinct sub-populations of cells. To understand this heterogeneity more fully we have performed mRNA transcriptome analysis of single cells across a wide sampling of phenotypically distinct populations. Using a automated system the capture, imaging, and routine preparation of full-length mRNA-sequencing libraries of single cells we have enabled the delineation of transcriptional heterogeneity within and between cell populations at the level of the individual cell. We present data that compares full length amplified transcriptome profiling by both high throughput gene expression using the BioMark HD system as well as downstream NGS sequencing of prepared cDNA libraries.

T-1014

THE IDENTIFICATION OF BRAIN METASTASIS INITIATING CELLS AND REGULATORS OF BRAIN METASTASIS FROM LUNG CANCER

Chitra Venugopal[1], Sara Nolte[1], Mohini Singh[1], Branavan Manoranjan[1], Nicole McFarlane[1], Olena Morozova[2], Robin Hallet[1], Erin O'Farrell[1], Naresh Murty[1], Edward Kachur[1], John Provias[1], John Hassell[1], Marco Marra[3], Sheila Singh[1]
[1]McMaster University, Hamilton, ON, Canada, [2]BC Cancer Agency, Vancouver, BC, Canada, [3]University of British Columbia, Vancouver, BC, Canada

Brain metastases (BMs) are most common in adults suffering from lung cancer, predicting uniformly poor patient outcome, with a median survival of only months. Despite their frequency and severity, very little is known about the tumorigenesis of BMs. We hypothesize that BMs contain a subpopulation of cells with stem cell properties, termed brain metastasis initiating cells (BMICs), that is responsible for the initiation of the brain metastasis. We applied our previously developed primary brain tumor-initiating cell (BTIC) models to the study of BMs from the lung to determine the presence of a stem-like population in BMs. Use of both patient samples and NCI-H1915, a lung to brain adenocarcinoma cell line, enabled us to determine useful strategies for study of BM.
We observed that brain metastases possess BMICs, and they have comparable sphere-forming capacity and stem cell frequency to primary brain tumor controls, are able initiate tumor growth, reproduce original tumor heterogeneity through in vivo differentiation, and can be serially passaged in vivo. We developed a BMIC xenotransplantation model, and injected BMICs into NOD-SCID mice via three routes (intracranial, intrathoracic to lung, and intravas-

cular) to assess for subsequent development, frequency and lethality of brain metastases. The minimally-cultured BMIC xenografts accurately recapitulated the patient tumor, and BMIC lines may also provide a suitable supply of cells when assessing the effects of new therapeutics on self-renewal or tumor growth.
We aimed to identify novel genes and/or pathways specifically overexpressed in brain metastasis tumorspheres. Transcriptome analysis obtained by RNA seq data was used to compare genes between brain metastasis tumorspheres, primary lung and primary brain tumors to determine genes overexpressed exclusively in the brain metastasis stem cell population. We identified 30 overexpressed genes that may have roles in lung-to-brain metastasis, including genes involved in cell adhesion, cytoskeleton rearrangements, proliferation/tumorigenesis, and formation/disruption of cell-cell junctions. To confirm their relevance to brain metastasis, we tested the predictive power of the candidate genes for overall patient survival, as poor survival of lung cancer patients strongly correlates with brain metastasis. We examined patient survival in a larger homogenous dataset of 226 primary lung tumors (GSE31210). Of the selected 28 candidate genes, 11 were associated with length of patient survival.
For the first time, we demonstrate the presence of a stem-like population in brain metastases from the lung. Our new model system provides a much needed method to study brain metastases from the lung, and is unique in its application of the CSC model to in vitro and in vivo assay development. Future studies will focus on elucidating the functional role of the selected genes overexpressed in the BMIC population. Our candidate genes may be essential to metastatic stem cell populations, where pathway interference may be able to transform a uniformly fatal disease into a more localized and treatable one.

T-1015

MIR-18B REGULATES SELF-RENEWAL OF GLIOBLASTOMA CANCER STEM-LIKE CELLS THROUGH NOTCH2, NEDD9, AND MEKK1

Xing Fan, Xiaobing He, Jason Heth, Karin Muraszko
Neurosurgery, University of Michigan, Ann Arbor, MI, United States

Glioblastoma (GBM) is the most common malignant brain tumor in human with extremely poor prognosis and new treatment for this deadly disease is desperately needed. Cancer Stem-like cells (CSCs) have been prospectively isolated from GBM and shown required for tumor propagation. We and others have demonstrated recently that differentiation of GBM neurospheres decreases GBM cell propagation both in vitro and in vivo (Zhu et al, Cancer Research, 71:6061-72, 2011). In the current study, we found that miR-18b was up-regulated upon differentiation of GBM neurosphere. When we introduced miR-18b into GBM neurospheres by lentivirus, we found that miR-18b decreases clonogenesis and growth of three different GBM neurosphere lines, indicating that GBM CSC population was reduced by miR-18b. Mechanistically, we found that miR-18b down-regulates luciferase activity of 3'-UTR reporters of NOTCH2, NEDD9, and MEKK1. Furthermore, protein expression of NOTCH2, NEDD9, and MEKK1 are also decreased by miR-18b, suggesting that NOTCH2, NEDD9 and MEKK1 are the direct targets of miR-18b in GBM CSCs. In addition, we have demonstrated previously that NOTCH regulates GBM CSC propagation through pAKT (Fan et al, Stem Cells, 28:5-16, 2010). Here, we found that pAKT and pERK1/2 were also down-regulated in miR-10b infected GBM neurosphere lines, indicating that miR-18b reduces GBM CSC propagation through NOTCH2/pAKT pathways. Taken together, we have demonstrated that miR-18b functions as a tumor suppressor and decreases GBM CSC propagation through targeting NOTCH2, NEDD9, and MEKK1, indicating that miR-18b can be a potential therapeutic molecule to target GBM CSCs and improve GBM treatment.

T-1016

INVOLVEMENT OF mTOR SIGNALING PATHWAYS IN REGULATION AND MAINTENANCE OF CANCER STEM CELLS OF GLIOBLASTOMA MULTIFORME

Dhruve S. Jeevan, Marissa D. Friedman, Michael Prendergast, Michael Tobias, Raj Murali, Meena Jhanwar-Uniyal
Neurosurgery, New York Medical College, Valhalla, NY, United States

Glioblastoma Multiforme (GBM) is a uniformly fatal primary brain tumor that renders poor prognosis despite current treatment modalities. Recurrence of tumor is attributed to the presence of treatment resistant cancer stem cells (CSCs) within the tumor. The hypothesis that tumors originate from CSCs has been strengthened by the findings that CSCs expressing specific markers isolated from the tumor mass of GBM are capable of generating tumors of the same genotype and phenotype within *in vivo* models. Treatment strategies' involving targeting of these CSCs is therefore crucial in the treatment of this disease.

One of the genes that appear to be responsible for tumor growth and progression is tumor suppressor gene PTEN, which acts via a canonical pathway of lipid kinase PI3K/AKT. Downstream of oncogene AKT is mechanistic target of Rapamycin (mTOR), which forms two multiprotein complexes, namely mTORC1 and mTORC2 that regulate cellular proliferation, growth, as well as migration. Recent studies have provided evidence that these complexes are also involved in CSC regulation. Moreover, the role of MAPK in differentiation of stem cells has been suggested. Here we delineate the role of mTOR/MAPK pathways in CSC maintenance and self-renewal, and further establish the means of targeting CSCs.

Our study demonstrates that a significant number of GBM tumors expressed activated $mTOR^{Ser2448}$ as well as stem cell marker Nestin. In culture, neurospheres derived from GBM cells expressed the markers of pluripotency (Musashi, Nanog, Nestin), the ability to differentiate into neurons (β-III-tubulin) and astrocytes (glial fibrillary astrocytic protein), and self-renewal capability. Genetic inhibition of mTORC components with the use of siRNA (Rictor, Raptor, or p70S6K) alters stem cell characteristics by influencing differentiation as well as maintenance of pluripotency. The differentiating agent all-trans retinoic acid (ATRA) was able to cause differentiation of neurospheres and reduce the levels of Nestin. mTOR or MAPK inhibitors influenced this effect. Proliferation of neurospheres, as measured by neurosphere diameter was suppressed by ATRA, Rapamycin (mTORC1 inhibitor) or U0126 (MAPK inhibitor) treatments. Further, self-renewal of CSCs was altered by mTOR/MAPK inhibition. While ATRA decreased neurosphere diameter by inducing differentiation, the inhibitors of mTOR and MAPK did so by suppressing the activation of down-stream target S6Kinase.

CSC differentiation and maintenance of pluripotency have clinical implications since these properties relate directly to the aggressive nature of GBM. Evidence that inhibitors of mTOR can influence CSC regulation suggests its potential therapeutic use.

T-1017

ESTABLISHMENT OF OSTEOSARCOMA CELL LINES ASSOCIATED WITH OSTEOID FORMATION AND LUNG METASTASIS BY USING PLURIPOTENCY-RELATED SMALL MOLECULES

Masaki Kawamata, Takahiro Ochiya
National Cancer Ctr Res Inst, Tokyo, Japan

It has been generally considered that establishing cancer cell lines while remaining their pathological characteristics is difficult because of a lack of effective culture methods. We have recently established rat embryonic stem cell (ESC) lines by adding 4 inhibitors YPAC that block ROCK, MEK, TGF beta and GSK3 signaling. YPAC could inhibit differentiation of the rat ESCs, leading to infinite proliferation while maintaining a state of inner cell mass of blastocysts. Here we use the YPAC medium to establish new cancer cell lines from an osteosarcoma that was spontaneously caused in a p53 heterozygous male rat. In this rat, lung metastasis was caused and osteoid formation was observed both in primary and the metastasized tumors.

YPAC medium allowed cancer cells of the primary tumor to adhere actively on collagen-coated dish, followed by continuous proliferation by passaging with enzymatic dissociation. However, cells could not proliferate under basic cell culture condition composed of 10% FBS in DMEM without YPAC (-i) nor 3i culture condition that have been generally used for rat ESC culture. In the osteosarcoma cell line cultured with YPAC (OSC+YPAC line), large population of cells kept alkaline phosphatase (ALP) activity of osteoblast marker after a long-term culture. Withdrawal of YPAC from the culture medium (OSC+YPAC to -i) induced cellular senescence and these cells lost ALP activity. To assess tumorigenicity, 3x106 cells of the OSC+YPAC or OSC+YPAC to -i cell line were transplanted subcutaneously to SCID mice. In 12 weeks, the OSC+YPAC cell line developed subcutaneous tumor with osteoid formation and such osteoid were metastasized to the lung. In contrast, OSC+YPAC to -i cell line did not show tumorigenicity. Here we succeeded

in establishing osteosarcoma cell line associated with lung metastasis and osteoid formation, which entirely reflect the pathological state of tumor spontaneously caused in a p53 mutant rat. We have demonstrated that YPAC is effective not only for establishing cancer cell lines but also for maintaining pathologic characteristics of tumors.

T-1018

XIAP INHIBITORS INDUCE DIFFERENTIATION AND IMPAIR CLONOGENIC CAPACITY OF ACUTE MYELOID LEUKEMIA STEM CELLS

Daniel Moreno-Martínez[1], Meritxell Nomdedeu[1], Marta Pratcorona[2], Marina Díaz-Beyá[2], Maria Rozman[3], Jordi Esteve[2], **Ruth M. Risueño**[1]

[1]Institut De Recerca Contra La Leucèmia Josep Carreras, Barcelona, Spain, [2]Department of Hematology, Hospital Clínic de Barcelona-University of Barcelona, Barcelona, Spain, [3]Department of Hematopathology, Hospital Clínic de Barcelona, Barcelona, Spain

Acute myeloid leukemia (AML) is a neoplasia characterized by the rapid expansion of immature myeloid blasts in the bone marrow. The course of the disease is marked by poor prognosis, frequent relapse, and high disease-related mortality. As such, new therapeutic approaches are required for remission induction and prevention of relapse. Due to the higher chemotherapy sensitivity and limited life span of more differentiated AML blasts, differentiation-based therapies combined with cytotoxic agents are a promising therapeutic approach. Based on public available gene expression profiles, a myeloid-specific differentiation-associated gene expression pattern was defined as the therapeutic target. An in silico screening was performed to identify small compounds that induce similar changes in the gene expression profile and a XIAP inhibitor (Dequalinium chloride, DQA) was validated. DQA, similarly to Embelin (another XIAP inhibitor), showed cytotoxicity against AML cells. Myeloid differentiation, downregulation of key signaling-mediator molecules and cell cycle arrest were observed after DQA treatment. Moreover, XIAP inhibition differentially impaired cell viability of the most primitive AML blasts and reduced clonogenic capacity of AML cells. Taken together, these results suggest that XIAP constitute a potential target for AML treatment and support the evaluation of XIAP inhibitors in clinical trials.

T-1021

IN VITRO COPMARISION OF A NOVEL HYALURONIC ACID-BASED DRUG DELIVERY SYSTEM AND FOLATE-RECEPTOR-TARGETED DELIVERY OF DOCETAXEL NANOPARTICLES AS TARGETED DRUG DELIVERY TO PROSTATE CANCER STEM CELLS

Reyhaneh Khoshchehreh[1], Navid Goudarzi[2], Mahdi Khoshchehreh[3], Rasoul Dinarvand[2], Marzieh Ebrahimi[1]

[1]1Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, Tehran, Iran, Islamic Republic of, [2]Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of, [3]Keck School of Medicine, University of Southern California, Los Angeles, CA, United States

Cancer stem cells (CSCs) possess high tumor-initiating capacity and have been reported to be resistant to therapeutics. It has been assumed that drug-resistant cancer cells may all be CSCs although the generality of this assumption is unknown. The engineering of drug-encapsulated targeted nanoparticles (NPs) has the potential to revolutionize targeted drug therapy in cancer. Here, we synthesized two different biofunctional polymers composed of HA:Cisplatin and PLGA-PEG-Folate. The physicochemical properties of the obtained nanoparticles were characterized by: dynamic
light-scattering analysis for their mean size, size distribution, and zeta
potential; and differential scanning calorimetry analysis for confirmation of molecular dispersity of cisplatin and docetaxel in the nanoparticles.
Nanoparticles were spherical with an average size of 200 nm in diameter and positive zeta potential values. For in vitro evaluation, prostate cancer cell lines were treated with these NPs, which led to populations of drug tolerant cells (DTCs). Surprisingly, these DTCs, when treated with docitaxel NPs, exhibited much reduced tumorigenicity

whereas HA-Cis treated cancer cells demonstrated no reduction in tumorregenerating capacity. DTX-NPs-treated cancer cells demonstrated low proliferative and clonogenic potential. Interestingly, DTX-NPTs-tolerant cancer cells showed decreases in many ‘‘stemness’’genes. Our results thus revealed that FOL-targeted NPs showed a high cytotoxicity effect in the prostate cell lines and showed a greater extent of intracellular uptake in FOL-receptor-positive cancer cells in comparison with the HA-Cis NPs.

T-1022

ACTIVATION OF CANONICAL WNT/BETA-CATENIN PATHWAY SUPPRESSES EMBRYONAL RHABDOMYOSARCOMA GROWTH AND SELF-RENEWAL BY DECREASING TUMOR PROPAGATING CELL NUMBER.

Eleanor Chen, Michael DeRan, Myron Ignatius, Ryan Clagg, Xu Wu, David Langenau
Massachusetts General Hospital, Charlestown, MA, United States

Embryonal rhabdomyosarcoma (ERMS) is a common soft tissue malignancy of childhood and phenotypically recapitulates fetal or regenerating muscle arrested at early stages of differentiation. The prognosis for patients with relapsed disease remains poor with 50% of patients succumbing to their disease. Thus, there is a clinical imperative to identify novel drugs that target relapse-associated cells. Work from our group has recently identified the tumor-propagating cell (TPC) in zebrafish ERMS that is responsible for driving relapse. The TPC is molecularly similar to a muscle satellite cell that expresses myf5, c-met, and m-cadherin. Targeting these cells for destruction would likely provide new therapies for the treatment of advanced RMS. Toward this end, we have successfully performed two independent chemical genetic screens using 47% of the FDA approved compounds and an additional 37,800 investigational compounds to identify drugs that suppress zebrafish ERMS growth in vivo by modulating the frequency of the TPCs and that elicit differentiation of human ERMS cells in vitro. From these large-throughput screens, GSK3-beta inhibitors, including BIO and lithium-chloride emerged as the most promising hits. GSK3-beta inhibitors lead to stabilization of beta-catenin and activation of canonical Wnt pathway. The role of canonical Wnt pathway in ERMS tumor progression remains poorly understood. In our study, BIO treatment of tumor-bearing zebrafish resulted in suppressed tumor growth by depleting myf5-GFP+ TPCs and in turn inducing differentiation of myf5-GFP+ TPCs into mature myosin expressing ERMS cells. More importantly, the myosin-positive ERMS cells lack self-renewing capacity as demonstrated by limiting dilution cell transplantation experiments. Specifically, treatment of BIO resulted in a 20-fold reduction in frequency of TPCs. We have now extended our findings to six clinically available GSK3-beta inhibitors, all of which have shown efficacy in suppressing human ERMS proliferation and in causing differentiation in vitro. A subset of these drugs is being assessed for efficacy in inhibiting growth of human ERMS in xenograft mouse models. In total, our work has identified the therapeutic potential of using GSK3-beta inhibitors for the treatment of relapsed RMS.

T-1023

CHARACTERIZATION OF TCF-DEPENDENT AND INDEPENDENT FUNCTIONS OF β-CATENIN IN COLORECTAL CANCER

Matthew G. Murphy[1], Maria Sol Flaherty[3], Ramanuj Dasgupta[2]
[A]Stem Cell Biology, [B]Cancer Institute, [1]NYU School of Medicine, New York, NY, United States, [2]City University of New York, NYCCT, New York, NY, United States

Misregulation of the Wnt/β-catenin signaling pathway is required for colorectal cancer (CRC), as mutations in the pathway are found in a vast majority of CRC patients. These mutations lead to an accumulation of β-catenin, which can translocate to the nucleus, where it interacts with transcription factors including the TCF/LEF family. Targets of β-catenin/TCF transcription include CyclinD1 and c-Myc, which function in cell growth and proliferation. The Wnt pathway has an important function in stem cells, as it has been implicated in both embryonic stem cells (ESCs) and cancer stem cells (CSCs). While much of the research on the Wnt pathway is focused on TCF-mediated transcription, β-catenin has been shown to interact with a number of other factors, including E-cadherin, which is critical for cell-cell adhesion, and pluripotency factors such as Oct4 and Klf4.

While β-catenin has been shown to be required in ESCs, evidence has shown that its interaction with TCF/LEF transcription factors is not. This lead us to investigate whether there is a similar requirement for β-catenin in CSCs which are responsible for tumor initiation. To investigate this, we used an *in vitro* sphere formation assay as a model for tumor initiation. We utilized a combination of TCF-transcriptional reporters, small molecule inhibitors, and inducible shRNA lines to assess the requirement of β-catenin/TCF transcription for maintenance of colorectal CSCs. We found that blocking the interaction between β-catenin and TCFs with small molecules developed by our lab reduces proliferation but does not prevent sphere formation or maintenance. In addition, we found a correlation between the expression of a TCF-transcriptional reporter and the ability of a single cell to form a sphere. Moving forward, we are translating our *in vitro* findings into an *in vivo* mouse model to determine the requirement for TCF transcription in tumor initiation.

T-1024

EVIDENCE SUPPORTING A CANCER STEMNESS HIERARCHY IN OVARIAN CANCER.

Brendan Ffrench[1], Louise Kehoe[2], Brendan Doyle[1], Aoife Cooke[1], Britta Stordal[1], Sharon O'Toole[1], Cara Martin[1], Orla Sheils[1], John O'Leary[1], Michael Gallagher[1]

[1]Histopathology and Morbid Anatomy, Trinity College Dublin, Dublin, Ireland, [2]Histopathology, Coombe Women's Hospital, Dublin, Ireland

Mouse tumorigenicity studies identify cancer stem cells (CSCs) as the
founding cells of tumors. Therapeutically targeting CSCs could remove the tumors malignant potential and circumvent chemoresistance, relapse and metastasis. The presence of multiple tumorigenic populations in tumors complicates CSC directed therapies. However, if multiple tumorigenic populations are part of a single stemness hierarchy it may be possible for a single therapeutic approach to target the entire hierarchy.
Six models of ovarian malignancy and one model of normal ovarian surface epithelium were screened for the presence of OvCSCs and somatic stem cells respectively. Three flow cytometry based CSC screens were implemented; ALDEFLUOR^{TM} (ALDH), Hoechst Side-Population and Cell Surface Protein (CD44, CD117, CD133, CXCR4) assays. Cells of interest were isolated via fluorescence-activated cell sorting (FACS). Putative CSCs (pCSCs) were validated in NOD.SCID mouse tumorigenicity assays (malignant potential) and single cell asymmetric division (AD) assays (differentiation and self-renewal potential).
The CSC screen identified pCSC populations ranging in size from 0.06 %
CD133+ (A2780 cell line) to 99.51 % CD44+ (59M cell line). All CSC screening assays identified pCSCs in one or more of the models screened. All models investigated contained pCSC populations marked by one or more assay. Multiple pCSC populations were identified within most cell lines. The sizes of these populations indicate that the pCSC populations do not overlap perfectly suggesting the possibility of hierarchical sub-populations of CSCs.
The ALDH+ pCSC and ALDH- non-pCSC populations identified in the A2780 and A2780cis cell lines were isolated and brought forward for i*n*
vivo and *in vitro* validation. Mouse tumorigenicity studies showed that both ALDH+ and ALDH- cells could produce xenograft tumors from 500 cells with equal efficiency. The AD assay showed that the ALDH+ cells were capable of differentiation and self-renewal. It also showed that the ALDH- population consisted of two cell types: i) ALDH_NegA cells, which can differentiate and self-renew to produce ALDH+ and ALDH- cells and ii) ALDH_NegB cells, which can not differentiate and only produce ALDH- cells. Further mouse tumorigenicity studies demonstrated that
ALDH_NegB also have a reduced malignant potential.
These results indicate the presence of an ALDH_NegA -> ALDH+ ->
ALDH_NegB cancer stemness hierarchy. It suggests that both ALDH_NegA and ALDH+ populations are tumorigenic. It is therapeutically advantageous to identify such hierarchies as it means that targeting a stemness hierarchy may be sufficient to remove the malignant
potential from the tumor as opposed to individually targeting multiple CSC populations.

Currently no ALDH_NegA/NegB markers have been identified. These populations can only be distinguished functionally. Microarray analysis is pending to identify such markers. Microarray analysis is also pending on 'undifferentiated' OvCSC populations and their differentiated counterparts to identify molecular targets to force differentiate these cells and to establish stable OvCSC models.

T-1025

THE REPRESSION OF NEGATIVE REGULATORS OF STEMNESS PATHWAYS IN LUNG CANCER

Erh-Hsuan Lin[1], Ting-Yu Kuo[1], Teh-Ying Chou[2], Yu-Chung Wu[3], Cheng-Wen Wu[1]
[1]Institute of Biochemistry and Molecular Biology, National Yang Ming University, Taipei, Taiwan, [2]Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, [3]Division of Thoracic Surgery, Department of Surgery, Taipei Veterans General Hospital and School of Medicine, Taipei, Taiwan

Stemness genes and pathways play important roles in embryonic development and adult tissue regeneration. Recent years, the aberrant activations of stemness signaling such as Hedgehog (HH), hypoxia-inducible factor (HIF), and Wnt pathways, and the stemness factors like Oct-4 and Sox-2 have been reported in lung cancers. However, most researches to date focused on the impact of positive regulators of stemness pathways in oncogenesis, but less on the importance of negative regulators. Here, using a mutant EGFR-mediated oncogenic transformation model in human lung epithelial cells, we found that Hedgehog interaction protein (HHIP) and HIF-3α, which are negative regulators of HH and HIF pathways, respectively, were significantly repressed in transformed cells. This observation was confirmed in several lung cancer cell lines and in human lung tumor samples. The repression of these genes was due to epigenetic silencing on their promoters. The overexpression of HHIP or HIF-3α significantly inhibited the colony formation, migration, and invasion abilities of lung cancer cells in response to stemness pathway activations. These results implicate the importance of EGFR-induced epigenetic alternations, and suggest that in lung cancer oncogenesis, the negative regulators of stemness pathways can be silenced and thus potentiate lung cancer cells to activate stemness signaling to acquire the abilities of survival, metastasis, and drug-resistance in adverse environments.

T-1026

SOX4 IS A MASTER REGULATOR OF EPITHELIAL-MESENCHYMAL TRANSITION (EMT) BY CONTROLLING EZH2 EXPRESSION AND EPIGENETIC REPROGRAMMING

Neha Tiwari[1], Vijay Tiwari[2], Lorenz Waldmeier[3], Piotr Balwierz[3], Phil Arnold[3], Mikhail Pachkov[3], Nathalie Meyer Schaller[3], Dirk Schübeler[4], Erik van Nimwegen[3], Gerhard Christofori[3]
[1]University Medical Center Mainz, Mainz, Germany, [2]Institute for Molecular Biology gGmbH (IMB), Mainz, Germany, [3]University of Basel, Basel, Switzerland, [4]Friedrich Miescher Institute for Biomedical research, Basel, Switzerland

Employing computational analysis of gene expression kinetics, we uncovered the transcription factor Sox4 with up-regulated activity during TGFβ-induced EMT in normal and cancerous breast epithelial cells. Sox4 is indispensable for TGFβ-induced EMT and cell survival in vitro and for primary tumor growth and metastasis in vivo. In addition to a number of EMT-relevant genes, Sox4 directly regulates the expression of Ezh2, encoding the Polycomb group histone methyltransferase that is known to trimethylate histone 3 lysine 27 (H3K27me3) for gene repression. Knockdown of Ezh2 expression prevents EMT, while forced expression of Ezh2 restores EMT in Sox4-deficient cells. Moreover, genome-wide analysis of H3K27me3 levels at consecutive stages of EMT revealed significant dynamics of H3K27me3 at the promoters of key EMT genes, and corresponding changes in their expression. Importantly, the epigenetic EMT signature predicts breast cancer patient survival. Our results provide a novel mechanism of EMT control by the transcription factor Sox4 which by governing the expression of critical EMT genes, as well as components of epigenetic machinery acts as a master regulator of EMT.

T-1027

KARYOTYPICALLY ABNORMAL HUMAN EMBRYONIC STEM CELLS ARE SENSITIVE TO HDAC INHIBITORS AND SHOW ALTERED REGULATION OF GENES LINKED TO CANCERS AND NEUROLOGICAL DISEASES

Lund Riikka J[1], **Maheswara Reddy Emani**[1], Barbaric Ivana[2], Kivinen Virpi[3], Jones Mark[2], Baker Duncan[2], Gokhale Paul[2], Harrison Neil[2], Nykter Matti[3], Lahesmaa Riitta[1], Andrews Peter W[2]
[1]University of Turku and Åbo Akademi University,, Turku Centre for biotechnology, Turku, Finland, [2]Department of Biomedical Science, The University of Sheffield, Centre for Stem Cell Biology,, Sheffield, United Kingdom, [3]Tampere University of Technology, Department of Signal Processing, Tampere, Finland

Genomic abnormalities may accumulate in human embryonic stem cells (hESC) during in vitro maintenance. Characterization of the mechanisms enabling survival and expansion of abnormal hESCs is important due to consequences of genetic changes for the therapeutic utilization of stem cells. Furthermore, these cells provide an excellent model to study transformation in vitro. We report here that the histone deacetylase proteins, HDAC1 and HDAC2, are increased in karyotypically abnormal hESCs when compared to their normal counterparts. Importantly, similarly to many cancer cell lines, we found that HDAC

inhibitors repress proliferation of the karyotypically abnormal hESCs, whereas normal cells are more resistant to the treatment. The decreased proliferation correlates with downregulation of HDAC1 protein, induction of the proliferation inhibitor, cyclin-dependent kinase inhibitor 1A (CDKN1A), and altered regulation of tumor suppressor protein Retinoblastoma 1 (RB1). Through genome-wide transcriptome analysis we have identified genes with altered expression and responsiveness to HDAC inhibition in abnormal cells. Most of these genes link to severe developmental and neurological diseases and cancers. These genes include factors linked to severe developmental disorders and neuronal disorders, such as Alzheimer's disease. Furthermore, most of these genes are linked to cancers, in particularly to those common in childhood. Our results highlight the importance of epigenetic mechanisms in the regulation of genomic stability of hESCs, and provide valuable candidates for targeted and selective growth inhibition of karyotypically abnormal cells.

T-1028

HOX GENES ARE UPREGULATED IN MALIGNANT COLONIC STEM CELLS AND ARE KEY TO THEIR SELF-RENEWAL

Seema Bhatlekar[1], Bruce Boman[1], Vignesh Viswanathan[1], Shirin Modarai[1], Lynn Opdenaker[1], Nicholas Petrelli[1], Jeremy Fields[2]

[1]Center for Translational Cancer Research, Helen F. Graham Cancer Center, University of Delaware, Newark, DE, United States, [2]CA TX Inc., Gladwyne, PA, United States*

Stem cells (SCs) regulating self-renewal pathways are critical for normal functioning of the colon whereas alteration of these pathways through cancer stem cells (CSCs) is key to colon tumorigenesis. The mechanisms of such alterations are not clear. HOX genes play a role in regulating stem cells (SCs) during embryologic development. Because HOX genes are aberrantly expressed in many cancers, we investigated the possibility that HOX genes are key to self-renewal of normal and malignant colonic stem cells. We hypothesized that (1) specific HOX genes are aberrantly expressed in CSCs in malignant colon and (2) this aberration is necessary for self-renewal of colon CSCs. PCR-based array analysis showed that most HOX genes are upregulated in colon carcinomas relative to normal colonic epithelium. Array results were validated using real-time PCR, western blots and immunohistochemistry. We discovered that HOXA4, HOXA9 and HOXD10 are expressed at the base of normal crypts, where most normal colonic SCs reside. Immunocytochemical analysis of SW480 and HT29 colon cancer cells showed up-regulation of HOXA4, HOXA9 and HOXD10. Using flow cytometry and Aldefluor assays, we isolated SCs and non-SCs from colon cancer cell lines and showed that HOXA4 and HOXA9 gene expression is increased in CSCs relative to non-CSCs. In normal SCs, HOXA4 and HOXA9 were co-expressed with SC markers ALDH1A1 and CD166. Colon carcinoma tissues also showed this co-staining and the number of co-stained cells was substantially increased relative to normal colonic epithelium. We studied functional roles of HOX genes in colon CSCs using treatment with retinoid agents and then sphere formation assays. Exposure of colon cancer cells to retinoid agonists induced down-regulation of HOXA4 and HOXA9 genes, decreased expression of the SC marker ALDH1A1, and reduced colonosphere formation. Taken together, our results indicate that over-expression of HOX genes is key to self-renewal of colon cancer CSCs, a mechanism that underlies growth of colon carcinomas. Strategies designed to target HOX genes may be a way to eradicate colon CSCs and may lead to the development of more effective therapies for colon cancer.

T-1031

CONVERSION OF HUMAN IMMORTALIZED CELLS TO CANCER STEM CELL-LIKE CELLS

Seung-ick Oh

Department of Biomedical Science, College of Health Science,, Korea University, Seoul, Korea, Republic of

Current models of stem cell and cancer biology assume that cancer stem cells (CSCs) reside at the apices of hierarchies, and are directly involved in the maintenance and progression of various types of cancer. Recently, it has been reported that a population of a primary tumor and oncogenes-expressing primary fibroblasts can reconstitute the original tumor on xenotransplantation. Here, we show that the human immortalized cell lines were converted

to CSC-like cells using our new *in vitro* culture system. We found that the CSC-like cells showed the expression of CD133, CD15, CD44, Nestin, NCAM, c-Myc and TERT, which have all been suggested to mark the CSC population. In our *in vitro* tumor-sphere formation experiments, the CSC-like cells efficiently formed tumor-spheres. In addition, our *in vivo* tumorigenecity experiments showed that only 10 cells of CSC-like cells were sufficient to initiate tumors in immunocompromised mice. These results showed the establishment of an experimental system to understand human CSC properties and suggested that pre-cancerous cells may have the potentials to be converted to CSCs.
Supported by the National Research Foundation (2012050131 and 20120007758) and the Ministry of Health and Welfare (A110606) Grants

T-1032

A NOVEL PRO REPROGRAMING FACTOR GLIS1 IS REGULATED BY HYPOXIA INDUCIBLE FACTORS VIA NON CANONICAL MECHANISM

Elham Khalesi[1], Keiji Tanimoto[2], Hideaki Nakamura[3], Andika Chandra Putra[2], Takahiro Fukazawa[1], Takeshi Imura[1], Yumi Kawahara[1], Yuichi Makino[4], Lorenz Poellinger[3], Louis Yuge[1]
[1]Department of Bio-Environmental Adaptation Sciences, Graduate School of biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan, [2]Department of Radiation Medicine, Research Institute for Radiation Medicine, Hiroshima University, Hiroshima, Japan, [3]Department of Cell and Molecular Biology, Karolinska Institutet, stockholm, Sweden, [4]Department of Medicine, Asahikawa Medical University, Asahikawa, Hiroshima, Japan

GLI-similar 1 (*GLIS1*) is a member of Krüppel-like zinc finger transcription factors that are closely related to GLI family. During mouse embryonic development, *Glis1* was expressed in a temporal and spatial manner; expression was prominent in several defined structures of mosodermal lineage, including craniofacial region, branchial arches, somites, vibrissal and hair follicles, limb buds, and myotomes. In adult mice, *Glis1* expression was observed abundant in placenta and adult kidney, and low in testis, thymus, colon, brain, and adipose tissue. Furthermore, significant induction of *Glis1* expression was observed in psoriatic epidermis and in mouse skin with the tumor promoter phorbor-12-myristate-13-acetate (PMA) treatment. However, the molecular mechanisms of regulation of *Glis1* expression in both physiological and pathological conditions have been mostly undetermined.
Glis1 was interestingly demonstrated to enhance markedly the generation of induced pluripotent stem cells (iPSCs) from both mouse and human fibroblasts when it is expressed together with *OCT3/4* (*POU5F1*), *SOX2*, and *KLF4*. DNA microarray analyses revealed that *Glis1* effectively promoted the direct reprogramming of somatic cells during iPSCs generation through activation of multiple pro-reprogramming pathways. It was also demonstrated that iPSCs generation was enhanced under hypoxic conditions, although underlying molecular mechanisms was still unclear. We thus examined whether hypoxia regulates *GLIS1* expression, and tried to clarify the molecular mechanisms behind this.
At first, variety of cell lines - MCF-7, MDA-MB-231, BT-474, SKBR-3, ZR-75-1 breast cancer cells; HSC-2, HSC-3, HSC-4, Ca9-22, KOSC-2 oral cancer cells; A549 lung cancer cells; HepG2 liver cancer cells; RCC4/pcDNA, RCC4/VHL kidney cancer cells - were incubated under normoxic (21% pO_2) or hypoxic conditions (1% pO_2) for 24 hours. Real-time RT-PCR analyses demonstrated that *GLIS1* expressed relatively higher in MCF-7 and RCC4 among cell lines tested. Interestingly, *GLIS1* expressions were dramatically increased in hypoxic conditions in some cell lines as well as known hypoxia-inducible genes, *ADM* and *CA9* and pro-reprogramming genes, *POU5F1* and *KLF4*. Importantly, *GLIS1* expression was significantly lower in VHL-transfected RCC4 cells than that in control RCC4. Furthermore, knock-down experiments demonstrated that inhibition of Hypoxia-inducible factors (HIFs) abolished hypoxic induction of *GLIS1* expression. Time course experiments showed that expression of *GLIS1* was gradually increased until 48 hours in hypoxic MCF-7 cells not only at mRNA but also at protein levels. A series of promoter analyses demonstrated that *GLIS1* transcription was regulated by hypoxia through HIF-dependent mechanism. Co-transfection analyses revealed that HIF-2[[Unsupported Character - Symbol Font ]] had more potent capacity on *GLIS1* promoter activation. Further analyses using wild-type, mutant types of HIF-2[[Unsupported Character - Symbol Font ]] lacking bHLH or C-terminal transactivation domains, or IPAS, demonstrated that protein-pro-

tein interaction was important but DNA binding was not necessary. These results suggest that the VHL-HIF pathway may play a pivotal role in regulation of the pro-reprogramming factor gene, *GLIS1*, *via* non-canonical mechanisms.

T-1033

CBFβ-SMMHC INACTIVATES P53 TUMOR SUPPRESSOR THROUGH ABERRANT PROTEIN INTERACTION AND RECRUITMENT OF HDAC8

Jing Qi[1], Sandeep Singh[1], Qi Cai[1], Ling Li[1], Hongjun Liu[1], Hieu Vu[1], Tinisha Mcdonald[1], Yinwei Ho[1], Chung-I Chang[2], ravi bhatia[1], Ya-Huei Kuo[1]

[1]hematopoietic stem cell & leukemia, City of Hope National Medical Center, Duarte, CA, United States, [2]Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan

Chromosomal inversion inv(16)(p13.1q22) is found in approximately 12% of acute myeloid leukemia (AML) patients, and leads to the fusion of the transcription factor gene CBFb and the MYH11 gene, and encodes a fusion protein CBFβ-SMMHC. Expression of CBFβ-SMMHC predisposes for leukemia transformation, however, the molecular mechanism underlying the leukemogenic function of CBFβ-SMMHC remains elusive. The tumor suppressor p53 is considered the master genomic guardian that is frequently mutated in a wide variety of tumors but is rarely mutated in inv(16) AML. Thus, we examined whether CBFβ-SMMHC fusion protein might impair p53 function. We found that p53 acetylation (Ac-p53) level was reduced in the presence of CBFβ-SMMHC fusion protein in the myeloid progenitor 32D cell line as well as in primary pre-leukemic bone marrow progenitor cells isolated from the conditional Cbfb-MYH11 knock-in (Cbfb56M/+/Mx1-Cre) mice. We showed that expression of p53 target genes are reduced in the presence of CBFβ-SMMHC fusion protein, indicating the p53 transcriptional activity was impaired, which is consistent with the impaired Ac-p53. To understand the mechanism, we found that CBFβ-SMMHC fusion protein interacts with p53 both in 32D cells and primary bone marrow cells. It has been reported that CBFβ-SMMHC interacts with histone deacetylase 8 (HDAC8) through the C-terminal SMMHC region, and we were able to detect a multimeric protein complex containing CBFβ-SMMHC, p53, and Hdac8. We further showed that Hdac8 knock-down led to robust increase in Ac-p53 levels while total p53 levels were modestly stabilized. In addition, treatment with HDAC8 remarkably increased Ac-p53To access whether HDAC8 plays a role in the deacetylation of p53, we used two independent small-hairpin (sh)-RNA to knock-down Hdac8 in 32D-CBFβ-SMMHC cells. To test whether this effect is dependent on the deacetylase function of HDAC8, we used HDAC8 selective pharmacological inhibitors (HDAC8i including PCI-34051 and 22D) directed against its catalytic sites. in both control and CBFβ-SMMHC cells. Since p53 protein levels were also increased upon HDAC8i treatment, we included Mdm2 inhibitor Nutlin-3 to stabilize p53. HDAC8i treatment alone or in combination with Nutlin-3 was able to enhance Ac-p53 compared to Nutlin-3 treatment, confirming its effect in restoring p53 acetylation. In order to test the function of HDAC8 in human stem progenitor cells (HSPCs), we treated HSPCs purified from normal human sample or inv(16) patient samples with HDAC8 specific inhibitor for 2 days, and result shows that the HSPCs from inv(16) samples are more sensitive to HDAC8 inhibitor compared to the normal samples, and the inhibitor is showing a selective induction of apoptosis from 5uM to 40uM. Collectively, our study shows that the CBFβ-SMMHC fusion protein forms an aberrant complex with p53 and HDAC8, leading to the aberrant deacetylation and impaired activity of p53. In addition, this deacetylation of p53 conferred by CBFβ-SMMHC is mediated by HDAC8. Our study reveals a novel leukemogenic mechanism in which CBFβ-SMMHC disrupts p53 activation through aberrant protein-protein interaction and recruitment of HDAC8.

T-1035

SEARCH FOR PATHOGENESIS OF ACQUIRED MYELODYSPLASTIC SYNDROMES USING REPROGRAMMING TECHNOLOGY

Kazuhisa Chonabayashi[1], Masahiro Kawahara[2], Akira Watanabe[1], Keisuke Okita[1], Masatoshi Nishizawa[1], Akifumi Takaori-Kondo[2], Shinya Yamanaka[1], Yoshinori Yoshida[1]

[1]*Department of Reprogramming Science, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan,* [2]*Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan*

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell diseases characterized by inefficient hematopoiesis and risk of progression to acute myeloid leukemia with poor prognosis. Although the incidence of MDS is relatively high, these diseases remain poorly understood, mainly due to a difficulty in the ex vivo culture of primary MDS cells and a lack of good animal models. Induced pluripotent stem cells (iPSCs) could act as a new platform for elucidation of the pathogenesis of MDS.
We used episomal methods to generate iPSCs from peripheral blood mononuclear cells of a MDS patient (RAEB-1 by WHO classification) with isolated 20q deletion. We established more than 30 iPSC lines derived from primary MDS cells (MDS-iPSC lines) as well as 6 normal iPSC lines derived from T cells (T-iPSC lines) of the same patient in parallel from the peripheral blood samples. Karyotyping and SNP-CGH analysis revealed that all MDS-iPSC lines have the chromosomal deletion (20q11.2-13.1) identical to those of the primary MDS cells, whereas all T-iPSC lines have normal diploid. All MDS-iPSC lines display characteristic morphology and expressed pluripotent stem cell markers at levels comparable to those in T-iPSC lines and ES cell lines. Five out of 7 selected MDS-iPSC lines formed teratomas so far.
We assessed hematopoietic differentiation potential of 7 MDS-iPSC lines and 6 isogenic normal T-iPSC lines using the OP9 co-culture system and the embryoid body differentiation culture system. Differentiation into hematopoietic stem cells (HSCs) of MDS-iPSC lines was comparable to T-iPSC lines. However, hematopoietic colony formation in methylcellulose culture and further differentiation in erythroid and neutrophil culture were severely impaired in all tested MDS-iPSC lines. Microarray analysis showed that 422 genes were up-regulated (fold change >2) and 308 genes were down-regulated (fold change <0.5) in MDS-iPSC-derived HSCs compared to T-iPSC-derived HSCs. In MDS-iPSC-derived HSCs, 46 genes located on 20q11.2-13.1 had reduced expression by at least 2 fold (74 genes by 1.5 fold) compared to the HSCs derived from the normal diploid T-iPSC lines. This iPSC-based system could be useful for studying the precise molecular mechanisms of MDS and testing new therapeutic compounds.

T-1036

ELITE MODEL FOR THE GENERATION OF INDUCED PLURIPOTENT CANCER CELLS (IPCS)

xueying wang
Biochemistry, National University of Singapore, Singapore, Singapore

Elite Model for the Generation of Induced Pluripotent Cancer Cells (iPCs)

Jason Lai[1], Chiou Mee Kong[1], Dashayini Mahalingam[1], Xiaojin Xie[1] & Xueying Wang[1].
[1]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.
[*Keywords:* iPCs; Stochastic Model; Elite Model; NSCLC; reprogramming]
Abstract
The inefficiency of generating induced pluripotent somatic cells (iPS) engendered two contending models, namely the Stochastic model and Elite model. Although the former is more favorable to explain the inherent inefficiencies, it may be fallible to extrapolate the same working model to reprogramming of cancer cells. Indeed, tumor cells are known to be inherently heterogeneous with respect to distinctive characteristics thus providing a suitable platform to test whether the reprogramming process of cancer cells is biased. Here, we report our observations that all randomly picked induced pluripotent cancer cells (iPCs) established previously do not possess mutations known in the parental population. This unanticipated observation is most parsimoniously explained by the Elite model, whereby putative early tumor progenies were selected during induction to pluripotency.

T-1037

INVESTIGATING THE ROLE OF MYF5 IN EMBRYONAL RHABDOMYOSARCOMA

Ines M. Tenente, Myron S. Ignatius, Eleanor Y. Chen, David M. Langenau

Molecular Pathology/Center for Cancer Research, Massachusetts General Hospital, Charlestown, MA, United States

Embryonal rhabdomyosarcoma (ERMS) is an aggressive pediatric sarcoma of muscle. Our group has recently shown that ERMS tumor-propagating potential is confined to a molecularly-distinct cancer cell type that expresses myf5 and additional satellite cell markers. Importantly, tumor-propagating cells (TPCs) drive continued tumor growth and are thought to drive relapse. Using a zebrafish transgenic model of ERMS, we have recently identified the myf5:GFP+/mylz2:mCherry-negative subpopulation as the TPC in this disease. Importantly, MYF5 is significantly upregulated in a majority of zebrafish, mouse, and human ERMS. Given that MYF5 has important roles in normal and regenerating muscle development, is highly expressed in muscle satellite cells, and is sufficient to reprogram human primary mesenchymal cells into muscle; we questioned if it exerts important roles in ERMS. We hypothesized that myf5 and its transcriptional targets are responsible for eliciting stem cell self-renewal programs within a subset of ERMS cells. To assess if myf5 is sufficient to drive ERMS self-renewal, we forced its expression in differentiated myosin expressing cells that normally lack tumor propagating potential. We find that myf5 expression confers self-renewal to this ERMS cell subpopulation. To assess if myf5 is required for tumor initiation, we have also generated ERMS in myf5-deficient zebrafish. Our data suggests that myf5 is important for ERMS initiation, as time to tumor onse is delayed when compared with fish that have intact myf5 signaling. In total, our data suggest important roles for myf5 in regulating ERMS growth and self-renewal.

T-1038

MICRORNA-30C REGULATES BREAST CANCER STEM CELLS AND EMT BY TARGETING TWF1 AND VIM

Huiping Liu[1], Jessica Bockhorn[1], Chika Nwachukwu[2], Simo Huang[1], Ya-Fang Chang[1], Aleix Prat[1], Dezheng Huo[2], Jun Lu[4], Charles Perou[5], Olufunmilayo I. Olopade[3], Geoffrey Greene[1], Michael Clarke[6]
[A]Ben May, [1]The University of Chicago, Chicago, IL, United States, [C]Medicine, [1]The University of Chicago, Chicago, IL, United States, [2]Yale University, New Haven, CT, United States, [3]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [4]Stanford University, Stanford, CA, United States

Our previous studies demonstrated that breast cancer stem cells (BCSCs) are actively involved in spontaneous metastases of patient tumor-derived human-in-mouse orthotopic breast tumor models. BCSCs have also been reported to mediate resistance to conventional chemotherapy and radiotherapy. However, the molecular mechanisms underlying BCSCs, metastasis and chemo-resistance are poorly characterized. We hypothesized that microRNAs link epithelial-to-mesenchymal transition (EMT) to BCSC-mediated metastasis and therapy resistance. Our work discovered that miR-30c, a human breast tumor prognostic marker, plays a pivotal role in linking metastasis and chemo-resistance by a direct targeting of cytoskeleton genes TWF1 and VIM, both regulators and markers of EMT. We also identified IL-11 as a secondary target of TWF1 in the miR-30c signaling pathway. Expression of miR-30c inversely correlated with TWF1 and IL-11 levels in primary breast tumors and low IL-11 associated with relapse-free survival in breast cancer patients. Furthermore, our study demonstrates that miR-30c is transcriptionally regulated by GATA3 in breast tumors. Identification of a novel miRNA-mediated pathway that regulates chemoresistance and apoptosis in breast cancer will facilitate the development of novel therapeutic strategies.
This study was supported in part by The University of Chicago Chicago Fellows Program, DOD W81XWH-09-1-0331, NIH K12 CA139160-02, NCI K99 CA160638-01A1, CTSA UL1 RR024999 (H.L.), Segal Fund and Ludwig Fund (G.L.G.).

T-1041

IDENTIFICATION OF LIN28A-INTERACTING RNAS WITH HITS-CLIP IN HUMAN BREAST CANCER CELLS

Masaki Mori[1], Peng Du[1], Robert Lapierre[1], Stephan Winklmeier[1], Fernando Camargo[2], Richard Gregory[1]
[A]The Stem Cell Program, [B]Division of Hematology/Oncology, [1]Boston Children's Hospital, Boston, MA, United States

LIN28A is an RNA binding protein that regulates stem cell pluripotency, embryonic development and growth, glucose metabolism, and tumorigenesis. A well-characterized molecular function of LIN28A is to selectively regulate

the expression of the let-7 family of microRNAs (miRNAs). Lin28A binds to the terminal loop structure of let-7 precursor miRNAs (pre-let-7) and recruits alternative 3' terminal uridylyl transferases (TUTases) Zcchc11 and/or Zcchc6 (also known as TUT4 and TUT7 respectively). These enzymes catalyze pre-let-7 oligouridylation that inhibits Dicer-mediated processing to the mature let-7 miRNA and promotes pre-let-7 degradation by an unknown nuclease. It is emerging that in addition to controlling let-7 expression LIN28A may have additional gene regulatory roles. Recent reports have identified a large variety of RNAs that associate with Lin28A in different cell types and it appears that Lin28A can either enhance or repress translation of some of the associated messenger RNAs (mRNAs). We previously found that Lin28A is highly expressed in the Her2-overexpressing subset of human breast tumors. Moreover, RNAi-mediated knockdown of Lin28A blocked the
tumorigenic capacity and metastatic potential of Her2-positive human breast cancer cells and xenografts. To gain further insight into the
oncogenic role of Lin28A in breast cancer and to identify possible new
therapeutic targets we sought to identify on a transcriptome-wide scale the
subset of RNAs that are bound by Lin28A in human breast cancer cells. To accomplish this we carried out LIN28A-HITS-CLIP (High-throughput sequencing of RNA isolated by crosslinking immunoprecipitation) using the T47D human breast cancer cell line to isolate RNAs associated with endogenous LIN28A protein. The RNAs we identified include some of the previously reported LIN28A-interacting RNAs such as let-7 miRNA precursors, and RPS13 a ribosomal RNA. This implies that LIN28A might regulate the altered metabolic, proliferative, and differentiation state of a tumor cell through LIN28-let-7 regulatory axis and ribosomal biogenesis. Interestingly our LIN28A-RNA interactome also uncovered previously unreported mRNAs, miRNA precursors, snoRNAs, transcripts of fusion genes, and long non-coding RNAs (lncRNAs). This analysis provides the first step towards a comprehensive view of LIN28A function in breast
cancer. Our most recent functional studies on some of these newly identified Lin28A-associated RNAs and relevance to breast tumorigenesis will be presented.

T-1042

LIN28 INTERACTS WITH SPECIFIC OCT4 TRANSCRIPT VARIANTS IN MEDULLOBLASTOMA CELLS

Marcia C T Santos[1], Patricia B G Silva[1], Fabio Pohl[1], Luiz O F Penalva[2], Oswaldo K. Okamoto[1]
[1]Bioscience, University of Sao Paulo, Sao Paulo, Brazil, [2]GCCRI Cellular & Structural Biology, UT Health Science Center, San Antonio, TX, United States

Neoplastic stem cells have been identified in a variety of human cancers, in which they are associated with the initial steps of tumorigenesis. The tumor-initiating cell phenotype may result from genetic alterations affecting the expression of critical genes regulating typical stem cell process such as self-renewal and pluripotency. The stem cell protein Lin28 is an RNA binding protein that controls the biogenesis of a group of miRNA, affecting their activity and stability, and expression of a subset of mRNAs at the post-transcriptional levels. LIN28 is ectopically expressed in some malignant tumors and highly expressed in undifferentiated ESCs and plays important roles in development, stem cell maintenance, oncogenesis and metabolism. Lin28 can be found in a variety of complexes including messenger ribonucleoprotein particles (mRNPs), polysomes, p-bodies and stress granules. In a previous study, aberrant OCT4 expression was correlated with poor survival of pediatric patients with medulloblastoma. In this study, Lin28 was found to be associated with RNPs containing OCT4 mRNA promoting its translation in medulloblastoma cells. The human OCT4 gene can potentially encode three spliced variants, designated as OCT4A, OCT4B and OCT4B1. Expression of all OCT4 isoforms was detected in medulloblastoma as in human embryonic stem cells. Interestingly, it was found that LIN28 associates specifically with OCT4A, but not with OCT4B and OCT4B1 transcript variants in medulloblastoma. These findings indicate that the specific interaction between LIN28 and OCT4A may activate pluripotency-related pathways in meduloblastoma cells thereby contributing to tumor aggressiveness.

T-1043

ABCB5 EXPRESSION IN COLORECTAL CANCER CIRCULATING TUMOR CELLS CORRELATES WITH METASTATIC DISEASE PROGRESSION.

Qin Guo, Jie Ma, Brian Wilson, Markus Frank, **Natasha Frank**
Children's Hospital Boston, Boston, MA, United States

Targeted personalized treatment approaches are critical for the development of successful therapies for advanced colorectal cancer (CRC). In this regard, circulating tumor cells (CTC) are of foremost interest as potential predictors of metastatic disease progression and patient therapeutic response. We have previously demonstrated that ATP-binding cassette member B5 (ABCB5) is expressed by cancer stem cells (CSC) in several human malignancies, including CRC, and that ABCB5 identifies therapy-refractory tumor subpopulations in CRC patients that correlate with disease progression. In order to evaluate the potential role of ABCB5 as a clinically relevant CTC marker, we utilized a human-to-mouse xenograft model system to examine the relationship of ABCB5-positive CRC-CTC to CRC metastasis. Human CRC tumor cells were grafted subcutaneously into immunocompromized NSG mice. 7 weeks after xenotransplantation, peripheral blood, lungs and liver were evaluated by qPCR for expression of human ABCB5 and GAPDH mRNAs. Expression analysis of these specimens revealed a significant positive correlation between peripheral blood human ABCB5 mRNA expression and aggregate metastatic disease load as determined by human GAPDH mRNA expression levels in lungs and liver ($r=0.8994$, $P=0.0147$). Remarkably, no significant correlation existed between peripheral blood human GAPDH mRNA expression and metastatic disease load, indicating a unique and essential role of ABCB5-positive CSC-CTC in colorectal cancer progression. Significantly increased peripheral blood ABCB5 expression was also observed in a clinical series of UICC stages I-III colorectal cancer patients ($n=105$) compared to healthy controls ($P<0.001$). Thus, our results identify the CSC antigen ABCB5 as a novel molecular marker of metastasis-associated CRC-CTC and point to potential utility of ABCB5 as a prognostic indicator and predictor of therapeutic response in patients with advanced colorectal cancer.

T-1044

CURCUMIN ATTENUATES TUMOR INITIATING STEM-LIKE PROPERTY OF HEAD AND NECK CANCER THROUGH MIR145 AXIS-MEDIATED PARACRINE SIGNALING

Shih-Hwa Chiou, Guang-Yuh Chiou
Department of Medical Research & Education, Taipei Veterans General Hospital, Taipei, Taiwan

Recent reports have demonstrated that head and neck cancer derived tumor initiating cells (HNC-TICs) presented high tumorigenic, chemo-radioresistant, metastatic properties, and coupled with gain of epithelial-mesenchymal transition (EMT) characteristics. As curcumin, an active component of the spice turmeric, can exert cytotoxic effects on cancer cells without harming normal tissues. The aim of this study was to investigate the chemo-therapeutic effect and regulatory mechanisms of curcumin on HNC-TICs. We first observed that the treatment of curcumin significantly down-regulated the ALDH1 activity, CD44 positivity, self-renewal property, and side population of HNC cells in a dose dependent manner. Using miRNA/mRNA-microarray analysis, curcumin significantly increased expression of tumor suppressive miR-145. Further mechanistic studies showed that the re-expression of PU-PEI-mediated miR145 delivery led to decreased of TICs properties. Blocking of endogenous miR145 can dramatically enhance stemness and tumor-initiating properties in ALDH1-CD44- non-TICs HNC cells. Additionally, the repressive effect of miR-145 on TICs properties was mediated by regulating of EMT. Importantly, in vivo nude mice model showed that cucumin treatment by oral gavage or PU-PEI-mediated miR145 delivery to xenograft tumors reduced tumor growth and metastasis and prolonged the survival times of tumor-bearing mice. From these results, we conclude that the inhibition of tumor aggressiveness in HNC-TIC in by curcumin was in part was mediated by up-regulation of miR-145, suggesting that curcumin would be a valuable therapeutics clinically in treatment modalities for malignant head and neck cancers by elimination of tumor initiating stem-like, and EMT properties.

T-1045

DNA DAMAGE RESPONSE AND STEMNESS PROPERTIES OF MURINE METASTASTIC TESTICULAR GERM CELL CANCERS

Amy M. Lyndaker, Timothy M. Pierpont, Claire M. Anderson, Donald H. Schlafer, Teresa L. Southard, **Robert S. Weiss**
Dept. of Biomedical Sciences, Cornell University, Ithaca, NY, United States

Testicular germ cell tumors (TGCTs) are the most common cancers of young men. Interestingly, advanced metastatic TGCTs are associated with a remarkably high survival rate following conventional chemotherapy, in contrast to most solid cancers. They are also distinct in that they typically retain functional DNA damage response (DDR) pathways, which could contribute to their responsiveness to genotoxic chemotherapies. To investigate the role of the DDR in the development and treatment sensitivity of TGCTs, we generated a novel mouse model featuring conditional inactivation of the Pten tumor suppressor and activation of the Kras oncogene in pre-meiotic spermatogonia using Cre/loxP recombination. Mice with both Pten deletion and Kras activation (Pten/Kras mice) developed teratocarcinoma, a mixed germ cell tumor containing both teratoma and embryonal carcinoma components. Consistent with these histopathological features, Pten/Kras-induced TGCTs had scattered clusters of cells that expressed Oct4, a pluripotency factor and marker of human embryonal carcinoma. While 70% of Pten/Kras mice developed TGCTs, typically by 4 weeks of age, only 16.7% of Pten single mutants and no Kras single mutants developed TGCTs, highlighting a potent synergy between Pten and Kras in tumor induction. Metastases containing teratoma tissue and in some cases Oct4-positive cells were identified in approximately one-fourth of Pten/Kras mice with primary TGCTs. Interestingly, early neoplasms in this model were nearly devoid of the DNA break marker γ-H2AX, suggestive of distinct DDR properties relative to other solid cancers. Continued analysis of this model holds great promise for elucidating how stem cell properties of the germ cells from which TGCTs arise impact DDR activity during tumor development and therapy, with important implications for the prevention and treatment of a variety of cancers.

T-1046

DRUG RESISTANCE CELLS OF COLON CANCER CELLS PRODUCE CARCINOMA EMBRYONIC ANTIGEN BUT ARE NOT CANCER-INITIATING CELLS

Yi-wei Huang, Chia-Ju Hsu, Li-yu Chen, Yu-Hsuan Chou, Akon Higuchi
Department of Chemical and Materials Engineering, National Central University, Jhongli, Taiwan

Tumors contain a small subpopulation of cancer-initiating cells, known as cancer stem cells (CSCs), that exhibit a self-renewing capacity and are responsible for tumor generation. CSCs are cells that can form tumors while having stem cell properties such as self-renewal and the ability to differentiate into multiple cell types. It is suggested that CSCs persist in tumors as a distinct population and cause relapse and metastasis by giving rise to new tumors. Specific surface markers for colon CSCs have been reported, and CD133 is the most studied surface marker for colon CSCs. Several other colon CSC markers have been proposed; these include: ESA, CD44, CD166, Msi-1, CD29, CD24, Lgr5, and ALDH-1. However, exact and reliable surface markers of colon CSCs have not yet been identified rationally. The only reliable method for identifying and quantifying CSCs is the observation of tumor formation in a serial xenotransplantation model. In this study, we evaluated the higher levels of CEA secreted by the LoVo colon carcinoma cell line, which was cultured in serum-free and serum-containing medium containing anticancer drugs. Drug-resistant LoVo cells were analyzed to determine whether those cells had CSC characteristics, e.g., small size of the cells/colonosphere and strong expression of CSC surface markers, as indicated by flow cytometry and immunohistochemistry analysis. Finally, in vivo tumorigenesis was examined by subcutaneously xenotransplanting the isolated drug-resistant LoVo cells into mice; we then evaluated whether the drug-resistant cells isolated in this study were CSCs. We found that drug-resistant cells, which comprised less than 1% of the LoVo human colon cancer cells that survived in serum-free or serum-containing medium supplemented with drugs (5-fluorouracil, acetylsalicylic acid, oxaliplatin, and cisplatin) were found to produce more than two orders higher than normal levels of carcinoembryonic antigen (CEA) per cell. These results raised the question of whether CSCs could be isolated from drug-resistant colon cancer cells when anticancer drugs are added to the culture medium. The percentage of cells positive for CD133, which is known to be a typical marker of CSCs, decreased in parallel with a decrease in the cell survival rate after the addition of anticancer drugs in both the serum-free and serum-containing media. Drug-resistant LoVo

cells had lower expression of CSC markers, including CD29, CD44, CD166, ALDH-1, Lgr5, and Msi-1, compared with the parental LoVo cells based on immunohistochemical examination. The drug-resistant LoVo colon cancer cells, selected by adding anticancer drugs to the culture medium, showed enhanced production of CEA in both serum-free and serum-containing media; these cells did not behave like CSCs in tumor generation experiments in vivo, even though CSCs have frequently been hypothesized to be drug-resistant.

Chromatin in Stem Cells

T-1051

EXPLORATION OF CHROMATIN INTERACTIONS IN PLURIPOTENT STEM CELLS

Hiroki Ikeda, Masamitsu Sone, Shinya Yamanaka, Takuya Yamamoto
CiRA, Kyoto Univ., Kyoto, Japan

Genome-wide analysis of histone modifications and electron microscopic observations have suggested that chromatin structures in somatic cell are reorganized during the reprogramming process. However, it remains unclear how chromatin structures affect pluripotency. There is a method which can elucidate the physically neighbouring regions of chromatin loci by proximity-based ligation of the DNA fragments in the interacted chromatin area, called 3C. Currently, by combining massively parallel sequencing, several methods have been invented based on 3C for detection of the genome-wide chromatin interactions. Here, we report the characterization of chromatin interactions in pluripotent stem cells determined by those various methods. First, we have studied higher order chromatin structures in the iPS cells using a 3C-based method, called Hi-C. As a result, we found that inter-chromosomal interactions in ES cells and iPS cells are more diverse than those in somatic cells. Moreover, we also found several different intra-chromosomal interaction areas between iPS/ES cells and somatic cells. To identify chromatin structures associated with the acquisition of pluripotency, we are now developing a new 3C-based method that can intensively analyze chromatin architectures of pluripotency genes and developmental key genes. So far, we detected different gene-loop formations between human iPSCs and somatic cells at several genomic regions (e.g. HOXA loci, etc.).

T-1052

SPATIOTEMPORAL CLUSTERING OF EPIGENOME REVEALS RULES OF DYNAMIC GENE REGULATION

Pengfei Yu[1], Shu Xiao[1], Wei Huang[2], Xiaoyun Xing[3], Chun-Xiao Song[4], Darina Mcdee[1], Tetsuya Tanaka[5], Ting Wang[3], Chuan He[4], Sheng Zhong[1]
[1]Bioengineering, University of California, San Diego, San Diego, CA, United States, [2]Institute of Mathematic and Statistic, Northeast Normal University, Changchun, China, [3]Department of Genetics, Washington University in St. Louis, St. Louis, MO, United States, [4]Department of Chemistry, University of Chicago, Chicago, IL, United States, [5]Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL, United States

Spatial organization of different epigenomic marks was used to infer functions of the epigenome. It remains unclear what can be learned from the temporal changes of the epigenome. Here, we developed a probabilistic model to cluster genomic sequences based on the similarity of temporal changes of multiple epigenomic marks during a cellular differentiation process. We differentiated mouse embryonic stem (ES) cells into mesendoderm cells. At three time points during this differentiation process, we used high-throughput sequencing to measure 7 histone modifications and variants including H3K4me1/2/3, H3K27ac, H3K27me3, H3K36me3, and H2A.Z, 2 DNA modifications including 5-mC and 5-hmC, and transcribed mRNAs and non-coding RNAs (ncRNAs). Genomic sequences were clustered based on the spatiotemporal epigenomic information. These clusters not only clearly distinguished gene bodies, promoters, and enhancers, but also were predictive of bidirectional promoters, miRNA promoters, and piRNAs. This suggests specific epigenomic patterns exist on piRNA genes much earlier than germ cell development. Temporal changes of H3K4me2, unmethylated CpG, and H2A.Z were predictive of 5-hmC changes, suggesting

unmethylated CpG and H3K4me2 as potential upstream signals guiding TETs to specific sequences. Several rules on combinatorial epigenomic changes and their effects on mRNA expression and ncRNA expression were derived, including a simple rule governing the relationship between 5-hmC and gene expression levels. A Sox17 enhancer containing a FOXA2 binding site and a Foxa2 enhancer containing a SOX17 binding site were identified, suggesting a positive feedback loop between the two mesendoderm transcription factors. These data illustrate the power of using epigenome dynamics to investigate regulatory functions.

T-1053

DIRECTED DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS IS ACCOMPANIED BY WIDESPREAD EPIGENETIC REMODELING AND REVEALS NOVEL PUTATIVE REGULATORY ELEMENTS

Casey Gifford[1], Michael Ziller[1], Cole Trapnell[1], Hongcang Gu[2], Alexander Tsankov[1], Julie Donaghey[1], David Kelley[1], John Rinn[1], Charles Epstein[2], Bradley Bernstein[3], Alexander Meissner[1]
[1]SCRB, Harvard University, Cambridge, MA, United States, [2]Broad Institute, Cambridge, MA, United States, [3]MGH, Boston, MA, United States

Differentiation of human embryonic stem cells (hESCs) provides a unique opportunity to study the epigenetic mechanisms that facilitate cellular transitions. To better understand the molecular basis of lineage specification, we performed comprehensive transcriptional and epigenetic profiling of three populations derived through directed differentiation of hESCs. Genome-wide mapping across these distinct populations, which resemble each of the germ layers found during embryonic development, for a combination of histone marks selected by the NIH Roadmap Epigenomics Project (H3K4me1, H3K4me3, H3K27me3, H3K27ac, H3K36me3, and H3K9me3), DNA methylation through whole genome bisulfite sequencing and RNA sequencing, reveals unique, lineage specific remodeling events. Most events are observed at distal regulatory sites associated with transcription factor binding, and often involve coordinated changes in both the DNA methylation level and enrichment of histone modifications, leading to three distinct epigenomic fates. Our data show gain of H3K27ac after differentiation at regions that have high DNA methylation levels in hESCs rather than H3K4me1 and H3K27me3. We additionally identify a novel dynamic, in which H3K27me3 is gained during differentiation in CpG poor regions that exhibit high levels of DNA methylation in hESCs. Interestingly, we find that this transition is associated with pioneer transcription factor binding sites, such as FOXA2, potentially elucidating an intermediate step in the decompaction of heterochromatin that occurs during the execution of cell fate decisions. Integration of these data sets allowed us to dissect the early events that dictate in vitro specification at high resolution, and in the future may help devise faithful differentiation protocols, leading to the in vitro derivation of therapeutically relevant tissue types.

T-1054

THE ROLE OF HP1α WITHIN HETEROCHROMATIN AND ITS EFFECT ON HUMAN PLURIPOTENT STEM CELLS

Marina Watanabe, Quan Zhu
Salk Institute for Biological Studies, SAN DIEGO, CA, United States

Pluripotent stem cells have the capacity to differentiate into all somatic tissues, and as such, are prized for their promising potential in tissue replacement therapy. Human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are the most commonly utilized pluripotent cells. Though often mistakenly considered equivalent, the two cell types have numerous differences including the presence of two varieties of iPS cells known as primed and naïve that differ in their pluripotency. The mechanisms behind the establishment and maintenance of pluripotency are not fully understood, but it is known that the epigenetic state of chromatin plays a critical part. Greater understanding of epigenetic mechanisms to control pluripotency and directed differentiation will likely improve their utility and manipulation, as well as interrogate the downstream usefulness of these important cell types in clinical applications. To determine the epigenetic differences among ES cells, primed, naïve iPS cells and somatic cells, we set out to investigate the dynamics and architecture of the silenced chromatin, the heterochromatin. Heterochromatin protein 1 (HP1), due to its role as a key heterochromatin regulator, was used as a marker to visualize

heterochromatin in live-cell and super resolution imaging beyond the optical diffraction limit at the 30nm level. Fluorescence Recover After Photobleaching (FRAP) experiments of HP1-GFP fusions indicate an increased exchange rate of HP1 proteins in hES cells compared to iPS cells, which are more dynamic than fibroblasts. Light sheet super resolution microscopy results suggest a more isotropic distribution of HP1 proteins in human ES and iPS cells than that in somatic cells. In addition, the results of these studies suggest a more open chromatin state in pluripotency with elevated heterochromatin dynamics.

T-1055

KDM2B MAINTAINS MURINE EMBRYONIC STEM CELL STATUS BY RECRUITING PRC1 COMPLEX TO CPG ISLANDS OF DEVELOPMENTAL GENES

Jin He, Li Shen, Ma Wan, olena Taranova, Hao Wu, Yi Zhang
Genetics, Pediatrics, Harvard Medical School, Boston, MA, United States

Polycomb group (PcG) proteins play important roles in repressing lineage-specific genes and maintaining the undifferentiated state of mouse embryonic stem cells (mESCs). However, how PcG proteins are recruited to their target genes is largely unknown. Here, we show that the H3K36-specific histone demethylase Kdm2b is highly expressed in mESCs and regulated by the pluripotent factors Oct4/Sox2 directly. Depletion of Kdm2b in mESCs causes de-repression of lineage-specific genes and induces early differentiation. The function of Kdm2b depends on its CxxC-ZF domain, which mediates Kdm2b's genome-wide binding to CpG islands (CGIs). Kdm2b interacts with the core components of the Polycomb repressive complex 1 (PRC1) and recruits the complex to the CGIs of early lineage-specific genes. Thus, our study not only reveals a novel Oct4/Sox2-Kdm2b-PRC1-CGI regulatory axis and its function in maintaining undifferentiated state of mESCs, but also demonstrates a critical function of Kdm2b in recruiting PRC1 to the CGIs of lineage-specific genes to repress their expression.

T-1056

ROLE OF INNATE IMMUNITY AND CHROMATIN REMODELING IN NUCLEAR REPROGRAMMING

Ji Eun Lee[1], Nazish Sayed[1], Kin Fai Au[2], Wing H. Wong[2], Renee Reijo Pera[3], John P. Cooke[1]
[A]Cardiovascular Medicine, [B]Department of Statistics, [C]Institute for Stem Cell Biology & Regenerative Medicine, [1]Stanford University, Stanford, CA, United States

Recently, we discovered that the viral vectors themselves, carrying the Yamanaka factors, play a critical role in nuclear reprogramming (Lee et al. Cell 2012). In seeking to develop reprogramming approach, we discovered a striking and consistent difference in the pattern of gene expression induced by viral versus protein-based delivery of the reprogramming factors. The difference in the pattern of gene expression suggested to us that a signaling pathway required for efficient nuclear reprogramming was activated by the retroviral, but not CPP approach. We confirmed, that the viral vector was more than a mere vehicle for genes encoding the reprogramming factors. In both gain- and loss-of function studies, we find that activation of toll-like receptor 3 (TLR3) plays a role in the efficiency of nuclear reprogramming. Stimulation of TLR3 causes rapid changes in the expression of epigenetic modifiers, with chromatin remodeling and changes in gene expression, which favor induction of pluripotency. To understand the epigenetic mechanism of innate immunity in reprogramming, we characterize the TLR signaling cascades and epigenetic modifiers that are activated during reprogramming using single cell mass cytometry together with DNA microarray analysis. Next, we determine the effects of innate immunity and the downstream epigenetic modifiers on chromatin modification. Using secondary MEFs that express a doxycycline-inducible cassette encoding the reprogramming factors, we determine epigenetic marks during iPSC derivation in the absence or presence of TLR activation using transcriptional and epigenetic profiling as well as functional assays. These studies highlight the gaps that remain in our understanding of nuclear reprogramming, and for the first time suggest a collaborative contribution of inflammatory pathways in the induction of pluripotency.

T-1057

ABERRANT METHYLATION SPREADING IS DICTATED BY CTG EXPANSION SIZE IN MYOTONIC DYSTROPHY-AFFECTED HUMAN EMBRYONIC STEM CELLS

Shira Yanovsky Dagan[1], Michal Avitzour[1], Moshe Shteiner[1], Gheona Altarescu[2], Paul Renbaum[2], Talia Eldar-Geva[3], Aharon Peretz[3], Stella Mitrani-Rosenbaum[4], Ephrat Levy-Lahad[2], Silvina Epsztejn-Litman[1], Rachel Eiges[1]
[A]Stem Cell Research Laboratory, [B]Medical Genetics Institute, [1]Hebrew University of Jerusalem, Jerusalem, Israel, [2]IVF Unit, Shaare Zedek Medical Center, Jerusalem, Israel, [3]Goldyne Savad Institute for Gene Therapy, Hadassah Hebrew University Medical Center, Jerusalem, Israel

Myotonic dystrophy type 1 (DM1) results from CTG repeat expansions within the 3`UTR of DMPK gene, aberrantly leading to DNA methylation of a CpG island. Here we characterized DNA methylation upstream of the CTGs in undifferentiated cells, explore its effect on local gene transcription and determined whether CTCF binding loss may be mechanistically involved. Using a wide range of 14 different human embryonic stem cells lines derived from DM1-affected embryos as well as DM1 affected iPS cells, we show that abnormal methylation is already established in pluripotent stem cells and is exclusively acquired by expansions of at least 300 CTG copies. The extent of methylation spreading from the upstream flanking region relies on expansion size; the larger the expansion, the more distant methylation extends towards the repeats. This association between expansion size and methylation extent is not detected if studying somatic cells of patients as it is restricted to uncommitted embryonic cells. Interestingly, we show that hypermethylation upstream of the repeats does not affect the expression of DMPK but rather strongly correlates with the reduction in SIX5, a nearby gene which is located immediately downstream to the repeats. In addition, we illustrate that the allele-specific reduction in SIX5 expression cannot be accounted for the binding loss of CTCF near the repeats, as formally suggested. This study emphasizes the power of mutant HESCs in advancing the understanding of the mechanisms underlying DM1 pathogenesis and, on a more general level, provides an excellent model system to study how CpG islands lose protection from DNA methylation under specific pathological conditions.

T-1058

REGULATION OF NEUROGENESIS AND GLIOMAGENESIS BY THE CHROMATIN REMODELDER CHD7

Weijun Feng, Muhammad Amir Khan, Pablo Bellvis, Olga Bernhardt, Haikun Liu
DKFZ, Heidelberg, Germany

Chromatin factors that regulate neurogenesis in the central nervous system remain largely unexplored. Here we demonstrate that the chromatin remodeler CHD7 (chromodomain-helicase-DNA-binding protein 7), a protein frequently mutated in human CHARGE syndrome, is a master regulator of neurogenesis in the mammalian brain. CHD7 is selectively expressed in neurogenic niches in the adult mouse brain, and inactivation of CHD7 in neural stem cells (NSCs) impairs neuronal differentiation and dendritic development. In line with this, CHD7 expression marks a class of human brain tumors with a molecular signature of neuronal differentiation. Strikingly, the CHD7 mutant phenotype in the hippocampal dentate gyrus is rescued by physical exercise. We further identified Sox4 and Sox11 as direct target genes of CHD7 that are responsible for its function in neurogenesis. Our study demonstrates an essential role of CHD7 in neuronal differentiation of NSCs, thus providing new insights into CHARGE syndrome and brain tumors.

T-1061

PHYSIOLOGICAL SIGNIFICANCE OF TET PROTEINS AND 5HMC IN PLURIPOTENCY AND DEVELOPMENT

Meelad M. Dawlaty, Rudolf Jaenisch
Whitehead Institute for Biomedical Research, Cambridge, MA, United States

Tet enzymes (Tet1/2/3) convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) in various embryonic and adult tissues. Although these enzymes have been implicated in promoting DNA demethylation, their physiolo-

gical significance is not well understood. While Tet3 knockout mice die at birth, mice mutant for either Tet1 or Tet2 are viable raising the question whether these enzymes have overlapping roles in development. To investigate the effects of combined deficiency of Tet proteins on pluripotency and development, we have generated a series of embryonic stem cell (ESC) lines and mice deficient for Tet genes individually or in combination. We find that while single deficiency of any of the three genes does not affect ESC self-renewal or pluripotency, combined loss of Tet1 and Tet2 leads to complete ablation of 5hmC in ESCs and causes developmental defects in chimeric embryos. Consistently, Tet1/Tet2 deficiency in mice, although compatible with development, is associated with increased embryonic abnormalities, perinatal lethality and reduced fertility. Our data show that both Tet1 and Tet2 contribute to 5hmC levels during development and their loss, though in part compensated by Tet3, promotes global hypermethylation and compromises imprinting. Moreover, the presence of substantial levels of 5hmC in double mutant embryos and adult mice suggests a significant contribution of Tet3 in hydroxylation of 5mC during development consistent with its role in promoting differentiation.

Pancreatic Cells

T-1063

COMPARATIVE EFFICACY OF HUMAN ADIPOSE TISSUE DERIVED STEM CELLS VERSUS STROMAL VASCULAR FRACTION IN PROTECTION OF ISLET SURVIVAL AND FUNCTION.

Jiyeon Lee, Songcheol Kim
Lab.of Cell Therapy,, Asan Medical Center, Seoul, Korea, Republic of

Islet cell transplantation is much simpler process compared with pancreas organ transplantation. However during process of isolating islets, islets were exposed to damage like mechanical and enzymatic digestion and oxidative stress.These process cause islet degradation and reduce the efficacyof islet cell transplantation.
Adipose tissue-derived stromal vascular fraction(ADSVF) contains multi cellular population like mesenchymal stem cells, endothelial precursor cells and immune regulatory macrophages. Adipose tissue-derived stem cells(ASCs) have immune modulatory properties, release growth factors and have the ability to differentiate into insulin producing cells. In this study, we examined the islet protection efficacy of ASCs and ADSVF *in vitro* and *in vivo*. ASCs and ADSVF were isolated from same donors. Islet were co-cultured with ASCs and AD-SVF. In FACS analysis, ASC and ADSVF showed different expression of surface antigen. The survival and function of islets were assessed by morphologically and by insulin secretion.

T-1064

COLONY FORMING CELLS IN THE ADULT MOUSE PANCREAS ARE EXPANDABLE IN MATRIGEL AND FORM ENDOCRINE/ACINAR COLONIES IN LAMININ HYDROGEL

Liang Jin[1], Tao Feng[1], Hung Ping Shih[2], Ricardo Zerda[1], Angela Luo[1], Jasper Hsu[1], Alborz Mahdavi[3], Maike Sander[4], David A Tirrell[3], Arthur D Riggs[1], Hsun Teresa Ku[1]
[1]City of Hope, Beckman Research Institute, Duarte, CA, United States, [2]University of California - San Diego, La Jolla, CA, United States, [3]California Institute of Technology, Pasadena, CA, United States, [4]University of California, San Diego, La Jolla, CA, United States

The study of hematopoietic colony-forming units (CFUs) using semisolid culture media has greatly advanced the knowledge of hematopoiesis. Here we report that similar methods can be used to study pancreatic colony-forming units (PCFUs). We have developed two pancreatic colony assays that enable quantitative and functional analyses of progenitor-like cells isolated from dissociated adult (2-4 months old) murine pancreas. We find that a methylcellulose-based semisolid medium containing Matrigel allows

growth of duct-like "Ring/Dense" colonies from a rare (1%) population of total pancreatic single cells. With the addition of roof plate-specific spondin 1, a Wnt agonist, colony-forming cells in Ring/Dense colonies can be expanded more than 100,000-fold when serially dissociated and re-plated in the presence of Matrigel. When cells grown in Matrigel are then transferred to a Matrigelfree semisolid medium with a novel laminin-based hydrogel, some cells grow and differentiate into another type of colony, which we name "Endocrine/Acinar". These Endocrine/Acinar colonies are comprised mostly of endocrine- and acinar-like cells, as ascertained by RNA expression analysis, immunohistochemistry, and electron microscopy. Most Endocrine/Acinar colonies contain beta-like cells that secrete insulin/C-peptide in response to D-glucose and theophylline. These results demonstrate robust self-renewal and differentiation of adult PCFUs-Ring/Dense *in vitro,* and suggest an approach to producing beta-like cells for cell replacement of type 1 diabetes. The methods described, which include microfluidic expression analysis of single cells and colonies, should also advance study of pancreas development and pancreatic progenitor cells.

T-1065

EPIGENETIC MODIFICATIONS ARE ASSOCIATED WITH ENHANCED PANCREAS DIFFERENTIATION FROM ENDODERMAL PROGENITOR CELLS

Lei Ying[1], Chenran Xu[2], Ken Zaret[2], Paul Gadue[1]

[1]The Children's Hospital of Philadelphia, Philadelphia, PA, United States, [2]University of Pennsylvania, Philadelphia, PA, United States

Diabetes is a healthcare burden effecting more than 25 million Americans. Diabetes mellitus type 1 which affects 15-20% of these individuals is characterized by a loss of insulin-producing beta cells of the pancreas, leading to insulin deficiency. While transplantation of cadaver islets does have therapeutic benefit, there is a severe shortage of donors. Therefore, in vitro genera functional β-cells from stem cell populations offer a possible alternative. However, embryonic stem (ES) cell-derived pancreatic-like cells typically produce poly-hormonal endocrine cells that are functionally unresponsive. Recently, our laboratory has described the generation of a novel endoderm stem cell population, termed endodermal progenitor (EP) cells, which can self renew and generate functional mono-hormonal beta-like cells in vitro. Recent studies have show that epigenetic modifications can prepattern foregut endoderm lineage specification, especially with regard to the H3K27me3 mark. We find that EP cells only gain the ability to generate mono-hormonal beta cells after several passages in culture, possibly due to establishment of epigenetic changes over time. In this study, we compared genome-wide gene expression profiles and CHIP-seq analysis of H3K27me3 on ES-derived definitive endoderm (DE) and high passage EP cells. Examination of pancreatic specific gene expression found little difference between these populations. Interestingly, we identified an enrichment of H3K27me3 on pancreatic-related genes specifically in EP cells. To determine if these differences have functional significance, we tested several small molecules that can either increase or decrease the H3K27me3 mark by inhibiting EZH2 or JMJD3 during different stages of pancreas differentiation from EP cells. Two EZH2 inhibitors, 3-Deazaneplanocin A (DZNep) and GSK-126, effectively increase the generation of insulin+ cells when given during the differentiation protocol. These treatments had no effect on the generation of mono-hormonal versus poly-hormonal cells. Our results underscored that modulation of epigenetic status can improve the efficiency of differentiation, which may provide a tool for generating functional and transplantable beta-like cells for translational research.

T-1066

PRODUCTION OF FUNCTIONAL ISLET CELLS FROM hIPSC: A STEP TOWARDS PERSONALIZED NUTRITION.

Filippo De Franceschi[1], Marine Kraus[1], Yuki Ohi[2], Anindita Bhoumik[2], Nicolas Bouche[1], Evert Kroon[2], Olivia G. Kelly[2], Emmanuel E. Baetge[1]

[1]Nestlé Institute of Health Sciences, Lausanne, Switzerland, [2]ViaCyte, Inc., San Diego, CA, United States

A renewable source of human beta cells would greatly benefit both fundamental research and cell therapy. Pioneering work by ViaCyte has shown that pancreatic progenitors can be differentiated from human embryonic stem cells in vitro; the subsequent transplantation of pancreatic progenitors cells in vivo results in functional beta cell production. ViaCyte has now extended their prior work by optimizing their recently published protocol (Schulz et al. 2012 PLoS One) for the differentiation of pancreatic progenitors from a panel of pluripotent stem cell lines, including induced pluripotent stem cells, yielding encapsulated functional beta cells in vivo (See ISSCR ViaCyte poster Y. Ohi et al.). Using these newly established methods, we utilized macro encapsulation technology to complete the differentiation of the IPSC-derived pancreatic progenitors in vivo (undifferentiated iPSC lines provided by Cellular Dynamics Inc.). The progenitors were loaded into devices manufactured with several different membrane types in order to evaluate the best setup for optimal cell survival, vascularization, and functional islet-cell differentiation. The cell-loaded devices were implanted subcutaneously in immunocompromised mice. Animals receiving the encapsulated grafts respond to glucose stimulation and produce human C-peptide (500-1000pM, 30 min post stim., 16 weeks post-engraftment). In summary, we have demonstrated that we can successfully reproduce the ViaCyte in vitro culture system for hiPS cell expansion and differentiation into pancreatic progenitors and in vivo maturation in macro-encapsulated devices into glucose responsive functional islet cells. These results enable development of IPSC-derived humanized pancreatic models for further investigation of genome Vs. function relationships.

T-1067

ALGINATE ENCAPSULATION AND DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS TO ISLET CELL TYPES

Thomas Richardson[1], Prashant N. Kumta[2], Ipsita Banerjee[1]

[A]Chemical Engineering, [B]Bioengineering, [1]The University of Pittsburgh, Pittburgh, PA, United States

Type 1 diabetes (T1D) is an autoimmune disease affecting a myriad of people worldwide wherein the beta cells of the pancreas are destroyed resulting in insulin dependence. The Edmonton Protocol of islet transplantation affords the feasible option for treatment of diabetes. However shortage of donor islets combined with immune rejection limits islet transplantation from becoming a viable therapy. We propose alginate encapsulation of insulin producing cells derived from human embryonic stem cells (hESC) as a potentially viable therapy for T1D, due to the virtually unlimited supply of hESCs and the immunoisolation capability of alginate capsules.

We developed a stage wise directed differentiation protocol to derive islet-like cells from hESCs. Definitive endoderm was induced with ActivinA and Wnt3Afor 4 days followed by pancreatic progenitor induction with Cyclopamine and Cyclopamine with Retinoic Acid for 2 days each, respectively. Maturation was induced by Nicotinamide for 2 days and Nicotinamide with DAPT for 7 days. A single cell suspension of ROCK inhibitor treated hESCs were suspended in 1.1% Ca alginate and 0.2% gelatin and encapsulation was achieved by drop wise addition into a bath of 100mM $CaCl_2$ with 10mM HEPES. hESCs were encapsulated at 3 different stages: after maturation to islet-like cells, at the definitive endoderm (DE) stage or as undifferentiated (UD) hESCs. The hESCs were continued with the remaining differentiation protocol based on the stage of encapsulation. Islet maturation was analyzed by gene expression of insulin, *MAFA*, glucagon, and *PDX1*. Further characterization was done by immunostaining, flow cytometry, and protein content analysis of islet-specific markers.

Our results to date show that encapsulation of fully matured cells result in low viability, as well as lower gene expression of mature markers compared to cells prior to encapsulation. However, when hESCs derived DE cells were encapsulated and further induced to islet like cells; the cells exhibited strong maturation markers, even higher than parallel tissue culture plastic (TCP) controls. The viability of the encapsulated cells, however, still remained low. Finally, we evaluated the feasibility of encapsulation of UD hESCs and allowing them to progress through all stages

of differentiation under encapsulation. The encapsulated UD hESCs remained viable and grew into large colonies towards the end of the differentiation protocol. While some dead cells were observed initially, large viable colonies were prominent towards the end of the protocol. Very encouragingly, many of the differentiation markers analyzed were even stronger when cells were encapsulated, in contrast to parallel control cells cultured in conventional TCP. To further analyze the maturation of hESCs fully differentiated under encapsulation, we measured the intracellular protein content of the islet-specific hormone c-peptide. The encapsulated cells showed 0.28pg c-peptide /ug total-protein, while TCP controls showed only 0.085pg c-peptide/ug total-protein.
Our results to date indicate that islet-specific maturation of hESCs is not only feasible option in the encapsulated 3D alginate capsule system but is also a configuration that appears to significantly enhance the maturation of hESCs. Further studies are currently underway to understand the mechanism contributing to significant enhancement as well as to test the in-vivo functionality of these cells in diabetic mice.

T-1068

ESTABLISHING A COLONY ASSAY FOR ADULT MURINE PANCREATIC PROGENITOR CELLS WITHOUT CONDITIONED MEDIA AND SERUM

Lena Wedeken, Liang Jin, Tao Feng, Hsun Teresa Ku
Diabetes and Metabolic Diseases Research, Beckman Research Institute of City of Hope, Duarte, CA, United States

In vitro colony assays are invaluable tools to study self-renewal and differentiation of progenitor cells from various tissues. We recently developed a colony assay that allows quantitative and functional analysis of adult murine pancreatic progenitor-like cells in culture. Dissociated single cells from adult pancreas are plated into a semisolid medium containing methylcellulose (a viscous material), Matrigel (for extracellular matrix components), conditioned media, fetal calf serum and growth factors (nicotinamide, exendin4, activin betaB and vascular endothelial growth factor). The semisolid medium restricts cell movement yet permits a cell to self renew, proliferate, differentiate and form a colony. Using this assay, we found that adult murine pancreatic progenitor-like cells are able to give rise to cystic colonies after three weeks in culture. The single cell that initiates and forms a colony is therefore termed a pancreatic colony-forming unit (PCFU). Adult murine PCFUs are able to self-renew and differentiate into cells resembling ductal, acinar and endocrine cells in our colony assay. However, the undefined components in the conditioned media and serum may complicate the examination of molecular mechanisms that govern the self-renewal and differentiation processes of these progenitor-like cells in future studies. We therefore set out to establish a better-defined, serum-free culture condition for our colony assay. We found that replacing the conditioned media and fetal calf serum with epidermal growth factor (EGF), R-Spondin1, Noggin and Serum Replacement (Invitrogen) in our otherwise unchanged assay allowed colony formation. Omission of EGF or nicotinamide abrogated colony formation, suggesting the survival and/or proliferation of PCFUs are dependent on these factors. This new culture condition allowed the differentiation of PCFUs towards all pancreatic cell lineages with R-Spondin1 and Noggin capable of favoring the formation of ductal and endocrine like cells over acinar like cells. In conclusion, this better-defined culture condition is an improvement of our existing pancreatic colony assay and will allow for studying mechanisms of self-renewal and differentiation of adult pancreatic progenitor cells towards endocrine beta cells in future studies.

T-1071

IN-VIVO MATURATION OF HUMAN EMBRYONIC STEM CELLS DERIVED ENDOCRINE PANCREAS IN PERFUSION-DECELLULARIZED PANCREAS

Saik-Kia Goh[1], Suzanne Bertera[3], Ipsita Banerjee[2]
[1]Univ of Pittsburgh, Pittsburgh, PA, United States, [B]Department of Chemical and Petroleum Engineering, [1]Univ of Pittsburgh, Pittsburgh, PA, United States, [2]Division of Immunogenetics, Department of Pediatrics, Children's Hospital of Pittsburgh, Pittsburgh, PA, United States

Introduction: Type I Diabetes affects over 1 million people in the United States. Embryonic stem cells (ESC) have emerged as an alternative cell source to transplanting donor islets owing to its virtually unlimited replicative capacity and the potential to differentiate into islet-cells. However, ESC derived islet-cells are currently limited by their immature phenotype and functionality. Success with maturation is only achieved after transplantation into animal model to promote full differentiation. Governed by the understanding that the pancreatic matrix plays a significant role during islet development and maturation, we hypothesize that three-dimensional (3D) extracellular matrix (ECM) derived from native pancreas will provide an optimal microenvironment for functional maturation of hESCs, and will serve as a suitable scaffold for implantation.
Materials and Method: Cadaveric pancreata were isolated from adult mice (n=8) and decellularized via detergent (0.5% SDS) perfusion. Resulting pancreatic matrix was characterized for acellularity, preservation of ECM and feasibility to transplant for *in vivo* use. For recellularization, β-cell (MIN6, $30x10^6$ cells) was first seeded into decellularized pancreas. Insulin gene expression was evaluated after 5 days of *ex vivo* culture in perfusion bioreactor (n=3). For hESC differentiation, decellularized pancreas was seeded with hESC-derived definitive endoderm (DE) cells ($30x10^6$ cells) and differentiated into pancreatic progenitor (PP) cells by sonic hedgehog inhibition (cyclopamine) and retinoic acid induction. The PP cell-laden scaffolds were then implanted under kidney capsule of immunodeficient mice for *in vivo* maturation. PP cells implanted without scaffold served as controls (n=3). The recellularized constructs were evaluated via quantitative RT-PCR and immunostaining for pancreatic maturation markers and human C-peptide respectively.
Results and Discussion: Perfusion-decellularization of pancreas with 0.5% SDS resulted in complete removal of cells. Characterization of the decellularized pancreas revealed preservation of ECM and 3D architecture. Implantation of decellularized pancreas induced tissue remodeling but did not elicit adverse immune response. MIN-6 cultured in pancreatic constructs demonstrated higher upregulation of INS1 and INS2 gene expression compared to MIN-6 cultured on fibronectin, collagen I and IV surfaces ($P<0.05$). Repopulation and *in-vitro* differentiation of hESC-derived DE cells in decellularized pancreas showed 52.7±10.8 fold increase in PDX1 compared to Matrigel ($P<0.05$). *In vivo* maturation under kidney capsule after 8 weeks supported full differentiation of the seeded hESC into C-peptide expressing cells. Immunostaining showed that engrafted cells were also positive for NKX6.1, PDX-1 and co-localized with human nuclear antigen staining – confirming the matured β-cells were derivatives from the implanted hESC seeded pancreas graft.
Conclusion: Perfusion decellularized pancreas promotes β-cell function, and provides a favorable microenvironment for pancreatic differentiation when seeded with hESC-derived DE cells. The cell-laden matrices can be transplanted *in vivo* and successfully matured into insulin-producing cells. Transplantation into immunodeficient mice with streptozotocin-induced diabetes is underway to evaluate the therapeutic potential of the hESC-derived endocrine pancreas to achieve normoglycemia.

T-1072

DERIVATION, DIFFERENTIATION, AND GROWTH OF DEFINITIVE ENDODERMAL CELLS FROM REPROGRAMMED DONOR FIBROBLASTS

Casey Roberts[1], Rebecca Ogle[2], Silvia Chen[2], Patrick Sachs[2], Roy Ogle[3]
[1]Regenerative Medicine, Eastern Virginia Medical School, Norfolk, VA, United States, [2]Regenerative Medicine Institute, LifeNet Health, Virginia Beach, VA, United States, [3]Health Sciences, Old Dominion University, Norfolk, VA, United States

Objective
The objective of this study was to differentiate, grow, and maintain the endodermal gene expression of induced pluripotent stem (iPS)-derived definitive endodermal (DE) cells in culture.
Methods
Induced pluripotent stem cells created from BJ neonatal fibroblasts (Stemgent) and F13 adult fibroblasts (from dermal tissue donated for research to LifeNet Health and reprogrammed by StemGent) were cultured on Matrigel (BD Biosciences) with TeSR2 medium (StemCell Technologies) until ready for differentiation. For induction into the

DE lineage, the iPS cells were maintained in DE medium (RPMI-1640, (Invitrogen), 11.1mM L-glutamine (Invitrogen), 1% ABAM (Invitrogen), 0.2% FBS (Gibco), 100nM IDE-1 (StemGent)) for 3 days. The iPS-derived DE cells then were cultured for 7 days in one of the following: DE Step medium (RPMI-1640, 11.1mM L-glutamine, 0.2% FBS (Invitrogen), 1% ABAM, and 50ng/mL Activin A), B27 medium (RPMI, 11.1mM L-glutamine, 1% ABAM, 1X B27, and 50ng/mL Activin A), or IDE-1 medium (RPMI, 11.1mM L-glutamine, 1% ABAM, 1X B27, and 100nM IDE-1). At the beginning and end of the 7 days post-differentiation culture, mRNA was extracted from the cells and assayed for pluripotency and DE gene expression by qPCR and compared to the original fibroblast and iPS cell lines.

Results

The BJ fibroblast and F13 fibroblast-derived iPS cells expressed pluripotency genes (Sox2, Oct 3/4, and Nanog) and the surface marker, Tra-1-81. After differentiating the cells with DE media, the cells' expression of pluripotency genes diminished compared to the iPS cells, while expression of genes associated DE, Sox 17, HNF4A, and FOXA2, increased. The DE cells also showed a decrease in ectodermal and mesodermal gene expression versus the embryoid body-derived cells.

The DE cells were cultured for one week in either DE Step medium or B27 medium, and the cells were assayed for expression of markers of pluriopotency, ectoderm, mesoderm, and DE. The DE Step medium and the B27 medium were found to maintain the DE gene expression by the differentiated cells for one week in culture, while suppressing expression of the genes associated with pluripotency, ectoderm, and mesoderm.

ConclusionThe B27 medium was able to maintain the differentiated DE-derived from BJ and F13 iPS cells. The BJ and F13 iPS cells were differentiated into DE cells with concomitant reduction in expression of pluripotency genes. The DE cells were able to maintain a steady state DE gene expression level for one week in culture, without an increase in gene expression of other cell types. The DE intermediary cells should prove useful for further differentiation into cell types derived from DE such as pancreatic β cells.

T-1074

INSM1 IS ESSENTIAL FOR THE MAINTENANCE OF MATURE PANCREATIC BETA CELLS

Shiqi Jia[1], Andranik Ivanov[2], Mathew Poy[3], Florian Paul[4], Matthias Selbach[4], Carmen Birchmeier[1]

[A]Developmental Biology/Signal Transduction, [B]Systems Biology of Gene Regulatory Elements, [C]microRNA and Molecular Mechanisms of Metabolic Diseases, [D]Cell Signalling and Mass Spectrometry, [1]Max-Delbrueck-Center for Molecular Medicine (MDC), Berlin, Germany

One goal of stem cell research is to generate medical replacement cells/organs for patients, for instance the pancreatic β cells. In vitro generated pancreatic β cells however are functional immature. A better understanding of the mechanisms of pancreatic β cells maturation is important for generating final replacement β cells. Insm1 (Insulinoma-associated 1), encodes a zinc finger protein, expressed in developmental endocrine and nerve systems and continuously expressed in mature endocrine cells. Null mutation of Insm1 is lethal, with a phenotype of no insulin positive β cells formation in pancreas, while the precursor cells are accumulated. In this study, we showed that Insm1 is essential for the maintenance of mature pancreatic β cells in vivo. Conditional mutation of Insm1 in pancreatic β cells at adult stage resulted an impaired regulated insulin secretion, which is similar as that in immature pancreatic β cells. We further showed the molecular mechanism of Insm1 function i.e. the gene expression pattern, the interaction factors and genome wide DNA binging. More details will be shown on site.

T-1075

MANIPULATING THE CELL FATE OF STEM CELL DERIVED PANCREATIC PROGENITORS USING MODIFIED RNA

Felicia Walton Pagliuca[1], Jeffrey Millman[1], Wataru Ebina[2], Derrick Rossi[2], Douglas Melton[1]

[1]Harvard Stem Cell Institute, Harvard University, Cambridge, MA, United States, [2]Immune Disease Institute, Children's Hospital Boston, Boston, MA, United States

Pancreatic β cells control mammalian metabolism through glucose-mediated insulin secretion. Since loss or dysfunction of these cells results in diabetes, new sources of β cells would provide a novel therapeutic option for treating diabetes and preventing its complications. Pluripotent stem cells are a promising new source of replacement tissue, if tissue-specific differentiation can be recapitulated in vitro. During pancreatic β cell development, pluripotent stem cells differentiate into increasingly specialized cells characterized by the expression of master regulators like Sox17 in definitive endoderm and Pdx1 in subsequent pancreatic progenitor cells. Although cocktails of small molecules and growth factors can induce a subset of key transcription factors in cells derived from human embryonic stem (hES) cells, this approach has not yet generated functional beta cells in vitro. Direct genetic manipulation with modified RNA has been suggested as an alternative strategy for controlling cell fate conversion. Herein we report the generation of methods to transfect various modified RNA transcripts encoding pancreatic transcription factors into stem cell-derived pancreatic progenitors in order to impose specific cell fate changes. These experiments have shown that a single, transient pulse of Ngn3 RNA can increase the efficiency of endocrine induction in ES-derived pancreatic progenitors by 300%. We have also identified transcription factors that act synergistically with Ngn3 in vitro, including Rfx6 and Hnf1a, to induce β cell fate. Additional studies have revealed that a short temporal window exists within current pancreatic directed differentiation protocols in which Ngn3 is optimally capable of inducing insulin-expressing β cells relative to other islet cell types. Further work evaluating whether transfection of ES-derived endocrine cells with transcription factors normally found in mature beta cells can improve their function in vitro and how these cells perform in vivo in animal models is underway. Taken together, these results suggest that modified RNA transfection is a powerful new tool to control hES-derived β cell fate.

Liver Cells

T-1081

HEPATOCYTE-LIKE CELLS GENERATED FROM MODIFIED MRNA-DERIVED HUMAN INDUCED PLURIPOTENT STEM CELLS

Anna-Karin M. Sjogren[1], Tommy B. Andersson[1], Gabriella Brolén[2]
[A]Cardiovascular and Gastrointestinal Innovative Medicines DMPK, [B]Discovery Sciences Innovative Medicines Assay Development, [1]AstraZeneca R&D, Mölndal, Sweden

Background: Hepatocytes derived from human induced pluripotent stem cells (iPSCs) may be an attractive alternative source of liver cells for cellular transplantation therapy, however safety is a major concern; requiring iPSCs that have been produced without the use of viral transgenes. It has been shown that modified mRNAs can be used to efficiently reprogram somatic cells into iPSCs that are non-viral and transgene-free. *Aim:* The aim of this study was to use modified mRNAs to produce human iPSCs, differentiate these into hepatocyte-like cells and define their hepatic functionalities. *Results:* We reprogrammed human foreskin fibroblasts into iPSCs by transfections with modified mRNAs encoding *OCT4*, *KLF4, SOX2, LIN28* and *c-MYC*, adopted the resulting iPSCs to grow as single cells in a monolayer and subsequently differentiated them into hepatocyte-like cells using a serum-free, three-step protocol. The hepatocyte-like cells exhibited inducible cytochrome P450 enzyme activity, displayed hepatocyte-like morphology, expressed hepatocyte specific genes and proteins and had the ability to store glycogen. *Conclusion:* The single-cell culture of modified RNA-derived induced pluripotent stem cells allows rapid production of large amounts of non-viral, integration-free hepatocyte-like cells, which makes it a promising approach not only for regenerative medicine applications, but also screening assays in the pharmaceutical industry.

T-1082

AN INDUCED PLURIPOTENT STEM CELL MODEL TO STUDY MECHANISMS OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) ASSOCIATED WITH PNPLA3 POLYMORPHISMS

Nidhi P. Goyal, Paulina Ordonez, Lawrence SB Goldstein
University of California, San Diego; Sanford Consortium for Regenerative Medicine, La Jolla, CA, United States

Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in the adult and pediatric population. NAFLD has significant public health implications as it has multiple associated co-morbidities including obesity, hypertension, type 2 diabetes, and cardiovascular disease, all of which increase the risk of premature death. NAFLD denotes a histological spectrum of disease ranging from macrosteatosis, excess accumulation of triglycerides in the liver, to steatosis with inflammation, to fibrosis. NAFLD can lead to hepatocellular carcinoma as well as end-stage liver disease ultimately requiring liver transplantation. NAFLD is a complex disease that has both environmental and genetic components. Genome-wide association studies (GWAS) have recently identified a polymorphism in the gene *PNPLA3* that has a strong association with risk and severity of NAFLD. The variant allele of *PNPLA3* is associated with more severe biochemical and histological abnormalities of NAFLD. The protein product of *PNPLA3*, or adiponutrin, is involved in lipid metabolism, but its exact function in humans remains unclear. The pattern of expression of adiponutrin is different in mice and humans, making it difficult to extrapolate findings from animal models. We are designing isogenic lines of human induced pluripotent stem cells (hIPSC) from a known genetic background with the variant and wild type homozygous alleles of *PNPLA3* using TAL effector nuclease (TALEN) technology. We have designed TALENs specific to the *PNPLA3* SNP and are generating lines of hIPSCs. We have adapted a protocol to induce differentiation of hIPSC to hepatocyte like cells (HLC) to test the hypothesis that polymorphisms of *PNPLA3* induce abnormal lipid processing as a potential initiating event in NAFLD. We will test this hypothesis by studying patterns of lipid metabolism and distribution in hIPSC derived HLCs with the wild type and variant alleles of *PNPLA3*, and the effects that aberrant lipid processing may have on cellular disposal and stress pathways. This approach translates a population-based GWAS into an *in vitro* human model to study the pathophysiology of NAFLD at a fundamental level. More broadly our work is an example of how the combined use of hIPSC technology and targeted genome editing can serve as a strategy to model complex sporadic diseases.

T-1083

PUTATIVE ISOLATION AND CHARACTERIZATION OF LIVER PROGENITOR CELLS FROM ADULT MOUSE

Bin Li, Craig S. Dorrell, Pamela S. Canaday, Carl Pelz, Markus Grompe
Oregon Stem Cell Center, Oregon Health and Sciences University, Portland, OR, United States

Hepatocytes are good candidates for liver failure rescue and liver reconstruction. However, due to the shortage of donor hepatocytes, liver stem cells, which can differentiate into hepatocytes, are in needed. To seek the liver stem cells in the normal adult liver is to be answered. In our lab, we have isolated clonal progenitor cells with cell surface markers MIC1-1C3, CD133 and CD26 from both normal and injured mouse liver. Here, in our study, by way of flow cytometry based antibody screening, we subdivided MIC1-1C3+CD133+CD26-CD45-CD31-CD11b- ductal liver non parenchymal cells (NPCs) with another cell surface marker. RNA sequencing data revealed novel stem cell expressing genes in this cell fraction and the inter action between progenitor cells and other cells in liver. Three dimensional organoid culture and single organoid assay demonstrate that this kind of cell has high proliferative capability, clonal forming efficiency and replating capability. In two-dimensional culture, the liver progenitor derived organoids demonstrated hepatocyte morphology. These liver progenitor cells will be used for transplantation into liver damaged immunodeficient mouse model to define the liver repopulation capability.

T-1084

EFFICIENT DRUG SCREENING AND GENE CORRECTION FOR TREATING LIVER DISEASE USING PATIENT-SPECIFIC STEM CELLS

Su Mi Choi[1], Yonghak Kim[1], Joong Sup Shim[2], Joon Tae Park[4], Rui-Hong Wang[5], Jun O. Liu[2], Chu-Xia Deng[5], Zhaohui Ye[3], Yoon-Young Jang[1]
[A]Department of Oncology, [B]Department of Pharmacology and Molecular Sciences, [C]Institute for Cell Engineering, [1]Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2]Department of Surgery and the McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD, United States, [3]Genetics

of Development and Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, United States

Patient-specific induced pluripotent stem cells (iPSCs) represent a potential source for developing novel drug- and cell- therapies. Although increasing numbers of disease-specific iPSCs have been generated, there has been limited progress in iPSC-based drug screening/discovery for liver diseases, and the low gene targeting efficiency in human iPSCs warrants further improvement. Using iPSC lines from patients with alpha-1 antitrypsin (AAT) deficiency, for which there is currently no drug- or gene- therapy available, we established a platform to discover new drug candidates and to correct disease-causing mutation with a high efficiency. A high-throughput format screening assay based on our hepatic differentiation protocol was implemented to facilitate automated quantification of cellular AAT accumulation using a 96-well immunofluorescence reader. To expedite the eventual application of lead compounds to patients, we conducted drug screening utilizing our established library of clinical compounds, the Johns Hopkins Drug Library, with extensive safety profiles. Through a blind large-scale drug screening, five clinical drugs were identified to reduce AAT accumulation in diverse patient iPSC-derived hepatocyte-like cells. In addition, using the recently developed transcription activator-like effector nuclease (TALEN) technology, we achieved high gene targeting efficiency in AAT-deficiency patient iPSCs with 25-33% of the clones demonstrating simultaneous targeting at both diseased alleles. The hepatocyte-like cells derived from the gene-corrected iPSCs were functional without the mutant AAT accumulation. This highly efficient and cost-effective targeting technology will broadly benefit both basic and translational applications. Conclusions: Our results demonstrated the feasibility of effective large-scale drug screening using an iPSC-based disease model and highly robust gene targeting in human iPSCs; both of which are critical for translating the iPSC technology into novel therapies for untreatable diseases.

T-1085

INDUCTION OF PLASTICITY IN CD14+ CELLS ISOLATED FROM HUMAN PERIPHERAL BLOOD.

Jashdeep Bhattacharjee, Barun Das, Asok Mukhopadhyay, Pramod Upadhyay
PDC, National Institute of Immunology, New Delhi, India

Stem cells are the class of undifferentiated cells which have the potential to differentiate to various cell types. In many clinical conditions, stem cells are used as a part of cell based therapy. In most of the cases the stem cells are being procured from human cord blood or bone marrow of healthy donor. This often leads to immune rejection of the grafted cells by the host immune system thereby compromising the health of the patients. Autologous transplantation would have been the best therapeutic alternative in such cases. Isolating therapeutically significant number of bone marrow stem cells from a patient may compromise his/her health condition. It is the need of the hour to find a class of cell which have relatively higher abundance than stem cells, convenient to procure and last but not the least, have the potential to differentiate to cells of different lineage.
In the current study, **CD14+** cells isolated from the human peripheral blood were reprogrammed to induce plasticity in them so that they acquire the potential to differentiate cells of different lineage. The reprogrammed cells, called **Reprogrammed Monocyte(RM),** shows **downregulation** of CD14 surface marker and **upregulation** hematopoietic stem cell surface marker such as CD34, c-kit. RM has also shown to express **Nanog,** an embryonic transcription factor.
RM was cultured in hepatocyte differentiation media to generate hepatocyte-like cell in vitro, termed as **Neo-hepatocyte**. After 11 days of culture, Neo-hepatocytes were examined for the presence of albumin and HNF4α by **qRTPCR** and **immune cyto-chemistry**. Neo-hepatocytes showed expression of **albumin** and **HNF4α**. Unlike CD14+ cells (Day 0) and RM, Neo-Hepatocytes were able to metabolize **7-pentoxy resorufin** to **resorufin** (PROD Assay) which indicates the expression of **P450** enzymes by these cells.
The in vivo differentiation capability of RM is currently underway.

T-1086

A SYSTEMS BIOLOGY APPROACH TO GUIDE DIRECT CONVERSION STRATEGIES AND ASSESS CELL IDENTITY

Samantha A. Morris[1], Patrick Cahan[1], Hu Li[2], Emma C. Fink[1], Chloe E. Reichel[1], James J. Collins[2], George Q. Daley[1]
[1]Boston Children's Hospital, Harvard Medical School, Boston, MA, United States, [2]Department of Biomedical Engineering and Center for BioDynamics, Boston University, Boston, MA, United States

Many human diseases such as heart failure, diabetes and neurodegenerative disorders result from the deficiency or dysfunction of critical cell types. Strategies for therapeutic tissue repair or transplantation entails the in vitro manufacture of clinically relevant quantities of defined cell types. The generation of otherwise inaccessible cells also permits disease modeling, toxicology testing and drug discovery in vitro. While engineered cells are often assayed functionally, the equivalence of the derived cells to in vivo cell types is not determined in a standard or comprehensive manner. To address this issue, we developed CellNet, a computational platform that classifies derived cells according to their transcriptional resemblance to a reference data set collated from a range of unique cell types and tissues. We reconstructed the context-dependent transcriptional regulatory networks underlying these cell types and tissues, allowing us to reveal precise regulatory nodes at which derived cells differ from their in vivo counterparts. We have used the platform to assess whether complete cell conversion has been achieved and to identify the cascade of regulatory events that characterize three distinct examples of fate conversion: reprogramming to pluripotency, direct conversion of B cells to macrophages, and direct conversion of fibroblasts to hepatocyte-like cells (HLCs). In experiments to replicate the transcription factor-mediated conversion of fibroblasts to HLCs, our application of CellNet revealed the existence of aberrant endoderm patterning in HLCs, and by identifying the regulatory nodes at which HLCs differ from primary hepatocytes, suggested a modified strategy to better recapitulate the target cell type, which we subsequently confirmed via in vitro functional assays. CellNet thus represents both an analytic and diagnostic algorithm for guiding direct conversion strategies and assessing the fidelity of conversion by detecting gene regulatory networks that define cell identity.

T-1087

PROTOPORPHRIN IX ACCUMULATION IN ABCG2-DEFICIENT LIVER IMPAIRS MITOCHONDRIAL DYNAMICS AND DIFFERENTIATION POTENTIALS OF HEPATIC STEM/PROGENITOR CELLS

Hsiao-Min Chang[1], Yu-Hsing Lin[1], Fang-Pei Chang[1], Chia-Rui Shen[2], Chao-Lin Liu[3], Wan-Yu Mao[1], Chia-Ning Shen[1]
[1]Genomic Research Center, Academic Sinica, Taipei, Taiwan, [2]Department of Medical Biotechnology and Laboratory Science, Chang Gung University, Tao-Yuan, Taiwan, [3]Ming Chi University of Technology, New Taipei City, Taiwan

The ATP binding cassette transporter ABCG2 are found to express in a wide variety of stem cells and in several somatic tissues including liver. Our recent work showed that ABCG2 is involved in regulating porphyrin homeostasis of embryonic stem cells and accumulation of protoporphyrin IX induced by ABCG2 inhibition affect self-renewal of ES cells. However, the physiological role of ABCG2 in somatic tissues and stem cells is largely unclear. Here, we generated ABCG2 knock mice and showed that ABCG2-knockout in hepatocytes elevated levels of protoporphyrin IX and impaired mitochondrial dynamics and function. Either treatment of 5-Aminolevulinic acid or functional blockage of ABCG2 transporter with fumitremorgin C (FTC), a specific and potent inhibitor of ABCG2, which elevated the cellular level of protoporphyrin IX, disrupted mitochondrial fusion/fission balance in hepatocytes. We assume impairment in mitochondrial dynamics affecting differentiation capability of hepatic stem/progenitor cells. We further demonstrated that hepatic stem/progenitor cells could be activated in mice treated 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC). And these activated hepatic stem/progenitor cells could therefore replenish damaged hepatocytes. However, we found that knockout of ABCG2 affected liver repairing mediated by hepatic stem/progenitor cells. The results suggest mitochondrial dynamics connected to differentiation of hepatic stem/progenitor cells and ABCG2 transporter protects liver from protoporphyrin IX accumulation which allows differentiation-driven mitochondrial remodeling during liver repair.

T-1088

GROWING SURROGATE LIVER IN A NEW SWINE MODEL OF LIVER DISEASE

Junji Komori[1], Paulo Fontes[2], Eric Lagasse[1]
[A]Pathology, [B]Surgery, [1]Univ of Pittsburgh, Pittsburgh, PA, United States

Stem cell therapy has been viewed as a promising alternative to organ transplantation. However, for some patients, orthotopic cell-based therapy directed at a diseased organ may not be feasible for many reasons, ranging from a possible lack of an appropriate environment in cirrhotic and fibrotic liver during end-stage disease to the lack of a thymus in complete DiGeorge Syndrome. Consequently, a critical component of cell-based therapy for these patients is to establish an optimal in vivo site for cell and tissue transplantation to restore organ functions. We have previously demonstrated that primary hepatocytes injected directly into a single lymph node (LN) will generate an ectopic liver and rescue a tyrosinemic mouse model from lethal liver failure. Furthermore, we have demonstrated that murine LNs provide a beneficial environment for ectopic transplantation of other cell types such as thymic epithelial cells and pancreatic islets. To validate the clinical relevancy and feasibility of generating an ectopic liver in patients with liver disease, we generated a swine model of liver disease. After total portacaval shunts and hepatectomy, an autologous population of hepatocytes isolated from the removed lobe was transplanted back in the LNs of the animal. 1-2 months later, autopsies were performed. Almost all of the injected LNs demonstrated hepatocytes engraftment with vascularization.
Here, we provide the first report describing the use of a LN as a site for liver cells transplant and demonstrating definitely visible engraftment in a large animal model. This approach is providing a new concept to use the LN as an in vivo bioreactor in which to regenerate functional organs.

T-1091

LIVER REPOPULATION IN A DIPHTHERIA TOXIN-INDUCED LIVER DAMAGE MODEL WITH HUMAN PROLIFERATIVE HEPATOCYTES

Ranran ZHANG[1], Yunwen ZHENG[1], Takanori Takebe[1], Tomonori Tsuchida[1], Bin Li[2], Yasuharu Ueno[1], Hideki Taniguchi[1]
[1]yokohama city university, yokohama, Japan, [2]oregon health and science university, oregon, OR, United States

Background
Hepatocytes transplantation was considered as an alternative therapy for liver dysfunction including acute liver failure and metabolic disorders; however, this promising method was impeded by donor cell shortage and validation. Since human adult hepatocytes with scarce cell source loss of in vitro proliferation capability and have unstable validation, the cost of human adult hepatocytes transplantation was improved; therefore, human proliferative hepatocytes including human fetal liver cells (hFLC) and human hepatic stem cells (hHSC) transplantation was set to be used for overcoming those issues with vividly in vitro proliferation property and low cost. This study introduced a novel mouse model of Albumin-TRECK/SCID (TRECK: Toxin Receptor Mediated Cell Knockout) to generate humanized livers and provide in vivo differential microenvironment for donor cells.
Methods and Results
1.5ug/kg DT (diphtheria toxin) was administrated into 6-week old mouse, mouse hepatocytes in albumin-TRECK/SCID mouse liver were ablated within 72hrs, after mouse GOT/AST, GPT/ALT were analyzed, a million of donor cells were transplanted into liver damaged mouse. About 6 weeks later, albumin-TRECK/SCID mouse with human heaptocytes was scarified for human albumin concentration detection, immunostaining, human gene analysis, replacement rate calculation and other assays. Showed both of the transplanted hFLC and hHSC were succeed in generating humanized livers by albumin-TRECK/SCID mouse, the chimerism in albumin-TRECK/SCID mouse reached as high as 90%. Comparing with hFLC transplanted humanized mouse group, the hHSC derived humanized mouse had higher human albumin secretion also with better induction and differentiation; the induction effect of human albumin expression could reach over 1.5x105-folds higher than donor cells, to hCYP3A4, hCYP2C9, hCYP2C19, the highest induction effect were about 2.2x105, 0.8x105, 1.3x105 folds separately.
Conclusion

We successfully constructed humanized liver in albumin-TRECK /SCID mouse with human proliferative hepatocytes like hFLC or hHSC, and this humanized mouse may prospectively bring more functional applications such as drug metabolism; drug to drug interaction and other in vivo or in vitro studies.

T-1092

DIRECT INDUCTION OF HEPATIC PROGENITOR-LIKE CELLS FROM MOUSE FIBROBLASTS BY REPROGRAMMING FACTORS

Maiko Higuchi[1], Norihisa Furukawa[2], Katsuhisa Tashiro[2], Kenji Kawabata[2], Kazufumi Katayama[1], Masashi Tachibana[1], fuminori Sakurai[1], Hiroyuki Mizuguchi[1]

[1]Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Japan, [2]Laboratory of Stem Cell Regulation, National Institute of Biomedical Innovation, Ibaraki, Japan

Efficient differentiation of functional hepatocyte-like cells from other somatic cells could provide not only a valuable system for drug cytotoxicity and metabolism screening, but also a potential source of hepatocytes for regenerative therapy. Induced hepatic differentiation has been previously achieved using induced pluripotent stem cells (iPSCs). However, these protocols require two processes including fibroblasts into pluripotent state using four reprogramming factors (Oct3/4, Sox2, Klf-4 and c-Myc) and then differentiating them into the desired hepatocytes. This requires time-consuming and is not so efficient. Here, we report direct induction of expandable hepatic progenitor-like cells from mouse fibroblasts without forming iPSCs.
For reprogramming studies, we used mouse embryonic fibroblasts (MEF) from alpha-fetoprotein (AFP)-GFP transgenic mouse embryos at day E13.5 to monitor conversion into the hepatic lineage. To induce partially reprogrammed state, AFP-GFP MEF were transduced with lentivirus encoding four reprogramming factors and then cultured in reprogramming media for 5 days. From day 6, these cells were cultured in chemically defined medium containing BMP4 and FGF4 for differentiation into hepatocytes. At day 14, after transduction with lentivirus vectors, a small number of GFP-positive cells were observed, whereas no GFP-positive cells were observed in the MEF transduced with control lentivirus vectors. To determine whether these cells were hepatocyte lineage or not, GFP-positive cells on 21 days after transduction were sorted and analyzed for gene expression of hepatocyte lineage markers. GFP-positive cells expressed not only hepatic lineage marker, such as AFP and ALB, but also cholangiocyte marker, CK7. However, more mature markers, ASGPR1 and TO, were not expressed in GFP-positive cells. After replating, many GFP-positive colonies appeared and could be expanded during several passages with expression of hepatic lineage markers. GFP-positive cells show significant self-renewal potential and the morphology was gradually changed into that of hepatocyte progenitors. Moreover, these hepatic progenitor-like cells could differentiate into more mature hepatocytes by elongating culture with the above-mentioned medium containing dexamethasone and HGF for 5 days. These cells showed multiple hepatocyte specific features; expression of ALB and E-cadherin and the abilities to uptake indocyanine green and to store glycogen.
In summary, we have shown that overexpression of four reprogramming factors in fibroblasts and cultivation with defined media for differentiation into hepatocytes can give rise to rapidly generate hepatic progenitor-like cells without going through iPSCs state. Moreover, induced hepatic progenitor-like cells showed significant self-renewal potential. Therefore, our protocol may offer a benefit to rapidly generate a large number of hepatocyte-like cells for clinical application as well as for drug screening assay.

T-1093

CYTOKINE-DIRECTED DIFFERENTIATION AND IN VITRO EXPANSION OF HEPATIC HIPSC AND HESC DERIVATIVES

Malte Sgodda, Jeannine Hoepfner, Susanne Alfken, Tobias Cantz
Hannover Medical School, Junior research group stem cell biology, Hannover, Germany

Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hIPSCs) hold great promise in regenerative medicine. Hepatic differentiated pluripotent stem cells might serve as hepatic transplants for metabolic or acute liver diseases. So far, hepatic differentiation efficiencies of hiPSCs and hESCs are well studied, but enrich-

ment of functional active hepatic derivatives and a sufficient homogenous population for transplantation purposes need further attention.
In our study, we aimed for an efficient protocol that is applicable to differentiate both, hESCs and hiPSCs into an expandable definitive endodermal progenitor lineage. We were using a cytokine- and small molecule-based protocol stimulating the WNT pathway for direct differentiation of hESC and hiPS cells into a definitive endoderm progenitor population. Next, we sorted CD117 (c-Kit) and CD184 (CXCR4) double positive cells by flow cytometry and replated these cells for further propagation in a co-culture system with mouse fibroblasts. These endodermal progenitor cells were passaged up to 20 doublings and analysed each passage. The differentiation status during the passages was determined by qRT-PCR for the endodermal markers SOX17, FOXA2 and by flow cytometry for the cell surface markers CD117 and CD184. Subsequent hepatic differentiation of the expanded endodermal cells was performed and early hepatic specification markers (hHEX, GATA4, AFP) as well as late hepatic markers (HNF4, Albumin, CYP1A1) were analysed by qRT-PCR. The number of hepatic precursors during expansion and differentiation was estimated using a lentiviral reporter construct expressing eGFP under control of the TTR promoter. Finally, metabolic functions such as urea production, CYP450 metabolism, and albumin secretion was analysed in these eGFP positive cells.
In conclusion, our modified protocol allowed the differentiation of human ESCs and iPSCs into proliferative endodermal progenitor cells. After sorting these cells, further propagation for several passages resulted in a maintained expression of endodermal marker genes and in a decrease of pluripotency-associated gene expression. The expanded cells can finally differentiate into hepatic cells, which we characterised by qRT-PCR and functional metabolic analyses. Further experiments need to reveal if the cells differentiated under these conditions were suitable for cell transplantation approaches *in vivo*.

T-1094

EPIGENETIC DYNAMICS OF JMJC-DOMAIN-CONTAINING DEMETHYLASES DURING THE DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS TOWARDS HEPATOCYTES

Tia Hackett, Benjamin Tschudy-Seney, Xiaocui Ma, Manny Garcha, Ahmad Hasan, **Yuyou Duan**
Internal Medicine, University of California Davis Medical Center, Sacramento, CA, United States

Histone methylation and demethylation play important roles in regulating gene expression, genome integrity, and epigenetic inheritance, which regulate cell fate and identity. Histone demethylation is performed by histone demethylases which contain petidylarginine deiminase, lysine specific demethylase (LDS, containing LSD1 and LSD2), and at least 30 members of the JmjC-domain-containing (JmjC) demethylases. In this study, we investigated the dynamic changes of JmjC demethylases, identified their specific histone targets and evaluated potential biological function during the differentiation of human embryonic stem cells (hESC) towards hepatocytes. hESC line H9 was induced to definitive endoderm (DE), then hepatic differentiation was initiated using our established differentiation protocol. The differentiated cells were harvested at days 2, 5, 8, 15, 22, 30, and 36; cDNAs were then generated, and cellular total proteins, nuclear proteins and histone proteins were extracted at all these time points. In addition, the supernatants were collected every other day from day 10. 30 pairs of primers were designed and synthesized to amplify the 30 members of the JmjC demethylase family. All primer optimizations were done using Hela cells, human hepatoma cells, and human primary hepatocytes. qPCR results showed that expression of most JmjC demethylase family members decreased during the differentiation process when compared to those at day 2 after differentiation. Only five JmjC demethylase member genes were highly expressed at different time points; they were Hairless at day 15, JHDM1D, JMJD2A and JARID1A at day 22, and MINA53 at day 30, and their proteins were detected by Western blots. Some of the specific histone targets, such as JHDM1D targets, H3K9me2 and H3K27me; JMJD2A target, H3K36me2; and JARID1A target, H3K4me2, were identified by Western blots. However, histone targets of Hairless and MINA53 were not found. Histone modification of H3K4 and H3K36 activates genes, and of H3K9 and H3K27 silences genes. Therefore, histone demethylations on H3K4me2 and H3K36me2 would appear to activate gene expression, and on H3K9me2 and H3K27me2 to silences gene expression during the process of the differentiation and maturation. Our results from both qPCR and ELISA showed that the expression of alpha fetoprotein (AFP, immature marker) was highest at day 14, and its expression continued to decrease to day 36. Albumin

(ALB, more mature marker) expression kept increasing to day 30 of differentiation. This indicated that the cells were somewhat differentiated during the first two weeks, then the differentiated cells developed a more mature phenotype by late time points. Based on the time-course of the differentiation, it appears that Hairless was potentially associated with hepatic differentiation, JHDM1D, KMJD2A and JARID1A were associated with early maturation, and MINA53 was associated with late maturation. Further investigations will be performed to determine how these five demethylases may regulate hepatic differentiation and how they are regulated during hepatic differentiation. Thus, these results potentially represent an initial step in revealing the mechanism involved in the regulation of hESC differentiation towards hepatocytes.

Intestinal/Gut Cells

T-1101

M-CELLS REQUIRE SPI-B AND ARE INDUCED BY RANKL IN CULTURED ORGANOIDS

Kerstin Schneeberger[1], Michal Mokry[1], Wim de Lau[2], Hans Clevers[2], Edward Nieuwenhuis[1], **Sabine Middendorp**[1]
[1]Pediatrics, University Medical Center Utrecht, Utrecht, Netherlands, [2]Hubrecht Institute, Utrecht, Netherlands

Microfold cells (M-cells) are specialized intestinal epithelial cells in the follicle-associated epithelium overlying the Peyer's Patches. M-cells deliver luminal antigens to immune cells and thereby form a necessary link between the gut lumen and the mucosal immune system. In vivo studies have shown that M-cell differentiation can be induced upon binding of Rank ligand (RankL) to its receptor Rank.
We have used an intestinal organoid culture system to further investigate M-cell differentiation. We show that upon incubation with RankL, small intestinal organoids differentiated into Annexin V and GP2 expressing M-cells. These cells were able to take up beads, confirming M-cell function. Furthermore, we show that RankL-induced M-cell development was dependent on the transcription factor SpiB. Organoids established from SpiB-deficient mice did not develop into functional M-cells upon incubation with RankL despite expression of Rank. These data show that Rank-RankL interaction results in upregulation of SpiB, which is crucial for M-cell development.
However, forced overexpression of SpiB in intestinal organoids did not induce M-cell development, suggesting that SpiB is not operating autonomously. Therefore, we are currently looking into the involvement of another transcription factor in the induction of the M-cell developmental program.

T-1102

MTOR MAINTAINS LGR5+ INTESTINAL STEM CELLS AND REGULATES SECRETORY INTESTINAL EPITHELIAL CELL DIFFERENTIATION THROUGH A WNT-INDEPENDENT MECHANISM

Leesa L. Sampson, Matthew Grogg, Yi Zheng
Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States

The highly conserved Target of Rapamycin (TOR) signaling pathway links external stimuli, such as growth factors, amino acids, and energy status, to cell growth and proliferation machinery, allowing cells to properly respond to fluctuating physiological demands. mTOR is vital for embryogenesis and in lower organisms, such as *Planaria* and *Drosophila*, intrinsically regulates the adult stem cell proliferative response to injury and diet. How mammalian stem cells sense and respond to external stimuli via the mTOR pathway is not completely clear; but recent studies in murine hematopoietic stem cells (HSCs) suggest that mTOR signaling regulates stem cell self-renewal. Elevated mTOR activity is frequently associated with various types of cancer, including intestinal adenomas, leading to the therapeutic use of mTOR inhibitors that inhibit tumor cell proliferation and metastasis, but rarely lead to remission. It has been demonstrated that the likelihood of colon cancer relapse positively correlates with increased *Lgr5*-positive crypt base columnar (CBC) ISC marker expression, a relatively well-characterized, rapidly-dividing ISC population that is responsible for replacing shed gut intestinal epithelial cells (IECs). Understanding how mTOR functions in the ISC population could provide insights into the physiology of intestine biology and mTOR targeting therapy. A

recent study from the Sabatini lab found p-S6, a downstream mTOR target, absent from the *Lgr5*-positive ISC population. While we have also observed this phenotype, we find that mTOR itself and its direct target p-4E-BP1 does not exhibit a restricted expression pattern. As such, it is possible that mTOR has an ISC intrinsic role in regulating self-renewal and differentiation.

Here we present the first *mTOR* ISC and IEC progenitor conditional deletion mouse model ($mTOR^{lox/lox}$ *villin-cre* or $mTOR^{\Delta IEC}$ for intestinal epithelial cell deletion) designed to investigate the intrinsic requirements for mTOR in ISCs and their progeny. We find that mTOR is surprisingly dispensable for murine intestinal development and organismal survival. While ≈5-15% smaller in body size and weight than wild-type mice, $mTOR^{\Delta IEC}$ mice are fertile and survive at the WT rate. Histology shows that $mTOR^{\Delta IEC}$ proximal small intestinal villi are lengthened, but ileal villi are shortened, correlating with regional differences in $mTOR^{\Delta IEC}$ crypt cell proliferation rates and villus tip-localized IEC apoptosis. While ISC-associated marker gene expression, including Lgr5, Ascl2 and Olfm4, are significantly reduced in the mutant, Wnt signaling activity, a major ISC-regulatory pathway, remains unaffected. An examination of the secretory IEC differentiation status reveals that $mTOR^{\Delta IEC}$ goblet cells remain present, but are smaller and stain comparatively weakly with Mucin2, indicating reduced mucus production. $mTOR^{\Delta IEC}$ Paneth cells, however, are drastically reduced both in size and number, while enteroendocrine cells exhibit a four-fold increase. *In vitro* studies reveal that isolated $mTOR^{\Delta IEC}$ crypts fail to form enteroids in culture but can be rescued by exogenous Wnt3a, further indicating that mTOR is dispensable for Wnt signaling. Overall our data indicate that mTOR intrinsically regulates Lgr5-positive ISCs and their differentiation program, but is not essential for intestinal epithelial homeostasis and function.

T-1103

SOX4 REGULATES PROLIFERATION IN THE MURINE INTESTINAL STEM CELL COMPARTMENT

Adam D. Gracz[1], Danny C. Trotier[2], Scott T. Magness[3]

[A]Cell Biology and Physiology, [B]Medicine, [C]Medicine and Cell Biology and Physiology, [1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Intestinal epithelial stem cells (IESCs) must balance proliferation and differentiation in order to maintain a rapid rate of physiological renewal. Sox factors are versatile regulators of cell fate and proliferation in a wide range of tissues, and are expressed in stem and progenitor cells of the intestinal epithelium. Here we utilize a conditional knockout mouse model to demonstrate that intestinal epithelial-specific loss of Sox4 leads to an upregulation of IESC biomarkers, as well as an increase in markers of proliferation and downstream targets of the Wnt pathway. Interestingly, while Sox4 knockout animals exhibit no hyperplastic phenotype in vivo, intestinal crypts isolated from Sox4-null epithelium exhibit enhanced growth in vitro, consistent with constitutively active Wnt signaling. Together, these results suggest that Sox4 is a negative regulator of Wnt signaling in vivo, and may act to mediate growth signaling between epithelial cells that can be compensated for by epithelial-mesenchymal crosstalk.

T-1104

MATURATION OF MAMMALIAN INTESTINAL EPITHELIAL PROGENITORS, IN VITRO AND IN VIVO

Robert P. Fordham[1], Shiro Yui[3], Alison Madgwick[1], Tetsuya Nakamura[3], Nicholas RF Hannan[2], Ludovic Vallier[2], Roger A. Pedersen[2], Mamoru Watanabe[3], Kim B. Jensen[4]

[A]Wellcome Trust/Medical Research Council Stem Cell Institute, [B]Anne McLaren Laboratory for Regenerative Medicine, [1]University of Cambridge, Cambridge, United Kingdom, [2]Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo, Japan, [3]Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Copenhagen, Denmark

The principle objective of this study was to define the maturation of intestinal epithelium from its foetal state, using three-dimensional in vitro cultures and orthotopic transplantation.
The mature intestinal epithelium is the most rapidly self-renewing tissue in adult mammals, consisting of differentiated villi and proliferative crypt compartments, where Intestinal Stem Cells (ISCs) reside. In mice at birth, the intestine is developmentally immature, lacking crypts and Paneth cells, the supportive cells of the adult ISC niche. The overall process of intestinal development is similar in humans, except that crypt formation occurs in late gestation. Therefore studying mouse intestinal development provides a model system to understand the events that occur in humans in utero.
Mature intestinal epithelium can be propagated from ISCs in vitro as three-dimensional 'organoids'. Here I describe the establishment and utilisation of long-term three-dimensional cultures from immature intestine, which exist as cystic epithelial spheroids, 'Foetal Enterospheres' (FEnS). FEnS represent a transient state in tissue maturation that can also be captured developmentally from human foetal tissue and pluripotent cells differentiated into posterior definitive endoderm.
FEnS are of primitive cellular composition, with distinct signalling requirements to adult organoids. Notably, they do not require exogenous stimulation of the Wnt pathway for their maintenance and do not contain secretory cells, including Paneth cells. However, treatment of foetal intestinal epithelium with Wnt3a and R-Spondin (a potentiator of Wnt signalling) is sufficient to induce in vitro maturation to adult-like organoids. These organoids contain Paneth cells and can subsequently be maintained without exogenous Wnt3a, indicative of complete functional maturation. Furthermore this data suggests that an increase in Wnt signalling in intestinal epithelial cells during postnatal development may drive tissue maturation in vivo.
In a complementary approach to assess the in vivo potential of immature intestinal epithelium, FEnS were transplanted into a mouse model of ulcerative colitis previously used to demonstrate the contribution of adult colonic organoids to crypt regeneration. Foetal-derived cells were able to adhere to the denuded lamina propria within three hours after transplantation and engraft into the regenerating colonic tissue by three days. By one week after transplantation, the foetal cells formed adult crypt-like structures, morphologically indistinguishable from host crypts and with evidence of goblet cell differentiation. These FEnS-derived crypts were maintained for over one month, with localisation of proliferative cells to the nascent crypt base and expression of colonic epithelial markers. Altogether, FEnS demonstrated an ability to engraft into the injured adult colon and contribute to de novo cryptogenesis during colonic epithelial regeneration. These findings have implications for the choice of transplantable cell type in regenerative therapies, suggesting that easily-expandable immature progenitors have the potential to mature in vivo and contribute to tissue repair.

T-1105

THE PLURIPOTENCY FACTOR LIN28 REGULATES STEM CELL HOMEOSTASIS, TISSUE REGENERATION AND TUMORIGENESIS IN MAMMALIAN INTESTINES

Ho-Chou Tu
Hematology/Oncology, Children's Hospital Boston, Boston, MA, United States

LIN28A and LIN28B are RNA-binding protein paralogs highly expressed in embryonic stem cells and in early developing tissues. Through blocking the terminal processing of let-7 miRNAs and regulating the translation of a number of mRNA binding targets, LIN28 controls an array of targets that mediate proliferation, pluripotency, differentiation and metabolism in embryonic stem cells. To explore the role of the Lin28/let-7 axis in adult tissues and how the perturbation of its function might lead to diseases, we generated an inducible Lin28a (iLin28a) transgenic animal, so that Lin28 can be overexpressed once treated with doxycycline. Strikingly, upon Lin28a induction, we observed a dramatic disruption of the epithelial structure in the small intestines and colon. This disruption is evidenced by a near-complete loss of terminally differentiated cells and aberrant enlargement of the proliferative crypts. Interestingly, the Lgr5-positive intestinal stem cells (ISC) are not affected by iLin28a, suggesting that the targeted population of Lin28a-induced crypt expansion may be the transit amplifying progenitor cells instead of the stem cells. Furthermore, we found that iLin28a animals are more susceptible to IR-induced intestinal damage. Supporting this finding, overexpression of let-7 miRNA protected animals from irradiation-induced Gastrointestinal Syndrome.

These results indicate a critical role of the let-7 miRNAs to maintain intestinal homeostasis, as well as injury repair and tissue regeneration. Indeed, the let-7 miRNAs are highly expressed both in the crypts and the villi, while a number of let-7 miRNAs are downregulated upon IR injury. Lastly, both LIN28A and LIN28B are highly expressed in a variety of human cancers. The molecular mechanisms by which Lin28a/b promote tumorigenesis can be partly explained by the indirect upregulation of let-7 targets, which include oncogenes such as cMyc, RAS, HMGA2 and Cyclin D1. To model the role of the Lin28/let-7 axis in tumorigenesis in vivo, we overexpressed Lin28a and LIN28B specifically in the intestinal epithelia and found that these animals developed aggressive intestinal adenocarcinoma. In summary, we propose the Lin28/let-7 axis as important players regulating both injury-induced tissue regeneration, as well as tumorigenesis. Exploration of specific molecules targeting this pathway will provide novel insights into potential therapeutics for cancer and regenerative medicine.

T-1106

MODULATION OF COLONIC STEM CELL MICRORNA EXPRESSION BY A CHEMOPROTECTIVE DIET

Manasvi S. Shah[1], Laurie A. Davidson[2], Eunjoo Kim[3], Jason M. Knight[4], Roger S. Zoh[5], Jennifer S. Goldsby[2], Evelyn S. Callaway[2], Ivan Ivanov[6], Robert S. Chapkin[2]

[1]Endocrinology, Boston Children's Hospital, Boston, MA, United States, [A]Program in Integrative Nutrition & Complex Diseases, [B]Departments of Cellular and Molecular Medicine, [C]Electrical Engineering, [D]Statistics, [E]Veterinary Physiology & Pharmacology, [2]Texas A& M University, College Station, TX, United States

Perturbations in adult stem cell dynamics are generally believed to represent an early step in colon tumorigenesis. Recently, several studies have demonstrated the role of miRNAs in the maintenance of colon cancer stem cells. However, to date, the effects of chemoprotective dietary components (omega-3 fatty acids and butyrate) on colonic stem cell non-coding miRNA signatures have not been determined, nor has the miRNA expression profile in colon stem cells been documented. Therefore, our overall goal was to better understand how a colonic stem cell population responds to environmental factors such as diet and carcinogen. With the identification of Lgr5 as a definitive marker for intestinal stem cells, we used the highly novel, recently described, Lgr5-EGFP-IRES-cre ERT2 knock in mouse model. The mice were injected with azoxymethane (AOM, a colon carcinogen) or saline (control) and fed a chemoprotective diet containing omega-3 fatty acids and fermentable fiber (fish oil + pectin) or a control diet (corn oil + cellulose). Single cells were isolated from the colonic crypt and three different populations of cells were collected via fluorescence activated cell sorting (FACS): Lgr5high (stem cells), Lgr5low (daughter cells) and Lgr5negative (differentiated cells). Expression of 384 mature rodent miRNAs was determined from total RNA isolated from the sorted cells, and 29 miRNAs in stem cells were significantly altered ($P < 0.05$) compared to differentiated cells. Specifically, three miRNAs, miR-125a-5p, miR-190 and miR-191, exhibited significantly ($P < 0.05$) higher levels in stem cells compared to daughter cells and differentiated cells. Notably, the “oncomiR” miR-21 was significantly upregulated in the corn oil + cellulose + AOM group (CCA) compared to fish oil + pectin + AOM group (FPA) in stem cells, but not in daughter or differentiated cells. Also, in stem cells, miR-19b, miR-26b, miR-27b and miR-203 were upregulated by CCA compared to FPA. However, in differentiated cells, miR-19b and miR-18a were significantly downregulated ($P < 0.05$) in CCA fed mice. These data indicate for the first time that select dietary cues can impact stem cell regulatory networks, in part, by modulating the steady-state levels of miRNAs. To our knowledge, this is the first study to utilize Lgr5+ reporter mice to determine the impact of diet and carcinogen on miRNA expression in intestinal stem cells. Supported by NIH CA168312, CA129444.

T-1107

A CENTRAL ROLE FOR STROMAL WNTS IN INTESTINAL STEM CELL SELF-RENEWAL

Zahra Kabiri[1], Gediminas Greicius[2], Steffen Biechele[3], Babita Madan[1], Alex Zhong[4], Hamed Zaribafzadeh[1], Edison Edison[1], Ralph M. Bunte[1], Bart O. Williams[4], Janet Rossant[3], David M. Virshup[1]

[1]Cancer and Stem Cell Biology, Duke-NUS Graduate Medical School, Singapore, Singapore, [2]National Cancer Center of Singapore, Singapore, Singapore, [3]Program in Developmental and Stem Cell Biology, The Hospital for Sick Children Research Institute, Toronto, ON, Canada, [4]Center for Skeletal Disease Research, Van Andel Research Institute, Grand Rapids, MI, United States

Aberrant Wnt/β-catenin pathway activity is associated with many diseases. Inhibition of the Wnt pathway has therapeutic promise but may adversely impact the self-renewal of stem cells in different adult tissues. Furthermore, it is hard to predict toxicity as Wnt-producing cells in normal and transformed tissues are poorly defined.
We used genetic and pharmacological approaches to inhibit Wnt secretion to assess the effect on the mouse small intestine, a tissue thought to be highly Wnt dependent. First, we generated mice with a targeted deletion of Porcn, a non-redundant membrane bound O-acyltransferase enzyme essential for all Wnt secretion, in gut epithelial cells. Surprisingly, while epithelial Wnts are believed to play crucial roles in the small intestine homeostasis, Wnt target gene expression, proliferation and differentiation was normal in the intact gut of the villin-Cre/Porcnflox mice. Confirming the essential role of Porcn, we found that Porcn-deleted epithelial cells, unlike wildtype cells, cannot form organoids ex vivo unless supplemented with either stromal cells or exogenous Wnts. Thus, epithelial Wnt secretion can compensate for the loss of stromal Wnts in ex vivo growth, but the epithelial Wnts are dispensable in the intact intestine.
We next investigated whether pharmacological Porcn inhibition, which targets both epithelial and stromal, affected epithelial cell proliferation in vivo. Indeed, mice treated with the Porcn inhibitor C59 had a dose-dependent decrease in the expression of Wnt/β-catenin target genes, and in intestinal proliferation, leading to markedly impaired small intestine homeostasis. Thus, global Wnt secretion is required for intestinal proliferation.
Taken together, the data confirm the central role of Wnt secretion in intestinal homeostasis, and suggest that the stromal layer has a major role producing these Wnts.

Lung Cells

T-1111

HIGHLY EFFICIENT LUNG PROGENITOR GENERATION FROM HUMAN iPSC BY SELECTIVE CHEMICAL SCREENING

Hongmei Mou[1], Anthony Arvanites[2], Lance Davidow[2], Kevin Lam[2], Karen Kotkow[2], Lee Rubin[2], Jayaraj Rajagopal[1]
[1]Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA, United States, [2]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States

We have recently published a step-wise method for the generation from human iPSC of NKX2.1+ multipotent lung progenitor cells without contamination with NKX2.1+ brain and thyroid progenitors. Such lung multipotent progenitors can be converted into NKX2.1+SOX2+ proximal airway progenitors and further into NKX2.1+p63+ airway basal stem cells (Mou et al., 2012) that are both progenitor populations for mature airway epithelium. However, the major obstacle preventing the actual development of human lung disease models using iPSC is the inability to produce with near purity lung progenitors and then subsequently into differentiated pulmonary epithelial cell types in vitro. In this work, we have optimized our differentiation protocol to produce human airway progenitors with high efficiency from human iPSCs by using unbiased chemical screens. We have identified 6 molecules from the Kinase Compound Set and 6 drugs from NIH Clinical Collection which produced statistically significant (more than 3 fold) increases in the percentage of NKX2.1+ lung progenitor cells. We found out that multiple compound hits target PI3K/mTOR, PKC and MEK1/2-related signaling pathways, suggesting that these biological pathways are actively involved in human lung organogenesis. By performing additive/synergistic experiments using our lead hits, we generate a robust, economically efficient and generally applicable protocol for a highly enriched population (up to 85% purity) of lung progenitors. The derived lung progenitors possess the characteristic lung markers and lack of neural and thyroid markers. Importantly, the lung progenitors can be matured into airway basal stem cells (NKX2.1+p63+) and further differentiated into physiologically active ciliated cells in vitro.

Our success of generation of functional airway progenitor cells with near purity is an important step closer to the utility of disease-specific iPS cells to model airway diseases like cystic fibrosis, asthma, bronchiectasis and lung cancer.

T-1112

HUMAN LUNG STEM CELLS AND THEIR POTENTIAL.

Feride Oeztuerk-Winder[1], Nicholas Bonenfant[2], Edgar J. Ruiz[1], Daniel J. Weiss[2], Juan-Jose Ventura[1]

[1]Wellcome Trust - Medical Research Council Cambridge Stem Cell Institute, Cambridge, United Kingdom, [2]University of Vermont College of Medicine, Burlington, VT, United States

Lung disease is the third most common cause of death in the United States and Europe. In many cases lung transplantation is the only definitive treatment, yet only 2700 lung transplants are performed each year, limited primarily by a lack of donor organs. The development of engineered lung tissue, which could be created using a patient's own cells, could have a significant impact on the treatment of end-stage lung disease.
We have isolated adult human lung stem cells (HLSCs), based on Lgr6/E-Cadherin expression, which culture indefinitely and can be considered as stem/progenitor precursors of alveolar epithelium (Oeztuerk-Winder *et al*., 2012). Optimized protocols enable *in vitro* differentiation and transformation of HLSCs, observing a matrix-directed lung cell specification in a variety of substrates (Matrigel, Fibronectin and Laminin). *In vivo* assays to investigate the cellular differentiation and self-renewal potential of HLSCs under physiological conditions included murine kidney capsule implants, which provide conditions that promote tissue differentiation.
We have shown that implanted HLSCs differentiate into various lung cell types *in vivo* and exhibit the same morphology as the lung, whilst also expressing lung tissue specific markers. The contribution of HLSCs to the repair of both human lung explants *in vitro* and murine lung tissue *in vivo* was also shown following bleomycin-induced injury. Cytokine and growth factor release, that are central to tissue remodelling, has also been demonstrated (Ruiz *et al*., submitted).
In order to engineer an artificial airway both a scaffold and stem/progenitor cells are required. The scaffold must provide the correct conditions for cell adhesion, proliferation and differentiation, as well as the transport of nutritional and growth factors. Decellularized lungs provide an opportunity to retain several key characteristics of the normal lung matrix and are superior to other matrix options for lung tissue engineering. We seeded Lgr6/E-Cadherin positive HLSCs into decellularized mouse or human lungs. They were then recellularized and differentiated into a alveolar type I and ATII epithelial cells in the lung.
In our work, we demonstrate initial progress towards the development of functional engineered lung tissue using rodent or human decellularized models. Besides the potential use in a clinical setting, engineered lung tissue can be used in the laboratory to study a wide variety of important aspects of lung biology and physiology.

T-1113

HIGHLY PURIFIED NKX2.1+ MOUSE ESC-DERIVED LUNG PROGENITORS DISPLAY FUNCTIONAL CAPACITY TO REPOPULATE 3-D DECELLULARIZED LUNG TISSUE.

Finn J. Hawkins[1], Tyler A. Longmire[1], Laertis Ikonomou[1], Sarah Gilpin[2], Harald Ott[2], Daniel Weiss[3], Darrell N. Kotton[1]

[1]Pulmonary and Critical Care, Boston University Medical Center, Boston, MA, United States, [2]Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA, United States, [3]Pulmonary and Critical Care, University of Vermont, Burlington, VT, United States

BACKGROUND: Two populations of Nkx2.1+ progenitors in the developing foregut give rise to the entire post-natal lung and thyroid epithelium. We demonstrated the directed differentiation and purification of primordial lung and thyroid progenitors derived from mouse embryonic stem cells (ESCs) by recapitulating normal early endoderm and lung development in vitro. A fundamental limitation in the field of lung regeneration is the lack of a platform to

assess the functional capacity of putative stem/progenitor cells. We sought to test the feasibility of using a recently described decellularized murine lung as a platform to assess repopulation potential and differentiation repertoire of ESC-derived stem/progenitor cells ex-vivo.

METHODS AND RESULTS: We developed a faithful lung epithelial reporter line with GFP targeted to the Nkx2.1 locus of mouse embryonic stem cells (ESCs) by homologous recombination. These ESCs were differentiated efficiently to anterior foregut endoderm using Activin-A followed by brief inhibition of BMP and TGF-beta. Key candidate growth factors suggested to specify lung lineages (e.g. FGF2, BMP4 and Wnt3a) led to efficient induction of Nkx2.1 by day 9 and on day 15 these Nkx2.1GFP+ were sorted to purity. With further expansion these purified cells expressed a global transcriptome that overlaps with developing lung and thyroid epithelium. To test the functional capacity of these putative lung progenitors, purified Nkx2.1 GFP+ cells were mixed with low melting temperature agarose and delivered by intra-tracheal instillation into decellularized murine lungs. The lungs were subsequently cooled and sectioned into 2-3mm thick slices and each slice was cultured in media containing Fgf2 and Fgf10 for seven days to promote expansion and further maturation of specified lung progenitors. The media was then changed to a fetal lung maturation media, supplemented with dexamethasone and cyclic AMP to augment the expression of lung epithelial genes, including surfactant protein C (Sftpc). Histology of these lung sections revealed that Nkx2.1 GFP+ cells were able to seed alveolar lung regions and adopt the morphology of lung alveolar epithelia. Approximately 70% of engrafted cells retained Nkx2.1 expression. Some engrafted cells acquired a flattened morphology, lost expression of Nkx2-1 protein and exhibited membrane associated expression of T1alpha, a pattern reminiscent of developing type I alveolar epithelial cells (AEC1). qRT-PCR of recellularized lung confirmed upregulation of additional markers of the developing lung including Sftpc, Sftpb and Scgb1a1 (CCSP). When sorted Nkx2.1GFPneg cells were seeded in an identical manner, there was virtually no engraftment observed. Control undifferentiated ESCs exhibited chaotic, clumped recellularization without significant expression of lung epithelial markers.

CONCLUSIONS

Purified mouse ES-derived Nkx2.1 GFP+ cells reconstitute a distal or alveolar phenotype when seeded in a 3-D decellularized murine lung scaffold.

T-1114

CD24 MARKS METASTATIC LUNG TUMOR-PROPAGATING CELLS WITH ACTIVE HIPPO SIGNALING

Allison N. Lau[1], Stephen J. Curtis[1], Samuel P. Rowbotham[1], Christine Fillmore[1], Morvarid Mohseni[1], Kerstin W. Sinkevicius[1], Zandra E. Walton[2], Juliana Barrios[1], Alexander Beede[1], Fernando D. Camargo[1], Kwok-Kin Wong[2], Carla F. Kim[1]
[1]Stem Cell Program, Boston Children's Hospital, Boston, MA, United States, [2]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, United States

Tumor-propagating cells (TPCs) contribute to cancer progression and metastasis. Our earlier studies found that the marker Sca-1 enriched for TPCs in murine lung tumors driven by oncogenic Kras and p53 loss. Here, we used this mouse model of lung cancer to probe the connection between tumor-propagating ability and metastatic capacity. To identify cell surface markers and further characterize TPCs, we conducted microarray analysis of Sca-1+ and Sca-1- tumor cells and found the marker CD24 was upregulated 3-fold (p=0.0057) in Sca-1+ cells. CD24 has been implicated in cancer progression, yet its role in metastatic lung cancer is lacking. Selection of CD24-expressing cells from primary murine lung tumors enriched for tumor-propagating activity and metastatic capacity in orthotopic transplantations. All recipient mice developed at least one lung tumor after transplantation of 10,000 CD24+ (13/13) or CD24- cells (14/14). 50% of recipients of 1,000 CD24+ cells developed tumors (3/6), whereas recipients of 1,000 CD24- cells did not exhibit lesions (0/3). The average number of tumors (14±6 vs 4.8±4.2, p=0.0019) and the average percentage of lung area filled with tumor (58%±23 vs 19%±20%, p=0.0017) were significantly greater in recipients of CD24+ vs. CD24- cells. Strikingly, there was an increase in metastases in recipients of CD24+ (11/12) vs. CD24- cells (6/12, p =0.03). CD24+ recipients developed metastatic lesions in the chest wall (11/11), local lymph nodes (4/11), and distant sites (4/11). CD24- recipients also had small metastases in the chest cavity (6/6) yet had limited development of local lymph node metastases (1/6) and no distant metastases (0/6). This demonstrated that CD24 prospectively marked metastatic tumor cells. In lung cancer cell lines, knockdown of CD24 decreased migration and reduced metastases post-transplantation. In transwell migration assays, shCD24 lines showed a 3-4-fold reduction in migration compared to shGFP cells (p<0.03). To assess metastatic capacity in vivo, shGFP and shCD24 cells were injected into the tail vein of nude mice. Transplantation of the shCD24 lines resulted in significantly fewer numbers of lung metastases after tail vein injection (p = 0.002). These findings strongly suggest that CD24 plays a functional role in TPC activity and metastasis. In an effort to evaluate other genetic programs that could participate in metastasis, we performed gene set enrichment analysis on the Kras;p53 TPC gene expression data. Two upstream regulators of the Hippo signaling pathway, Nf2 and Lats1, were part of the core enrichment in many of the top gene sets. Knockdown of the Hippo pathway mediators Yap and Taz decreased migration of murine lung cancer cell lines, (3.8-10-fold compared to shGFP, p<0.05) supporting a role for this pathway in lung cancer progression. These Yap/Taz knockdown cell lines were injected via tail vein to assess metastatic capacity in vivo. Metastases were less frequent in recipients of shTaz cells compared to shGFP (p<0.05). Our data suggest a novel role for CD24 and the Hippo pathway in regulating tumor-propagating cells and metastasis. Our characterization of aggressive TPCs within lung tumors has defined a subset of cells involved in metastatic lung cancer and the interruption of CD24 and Hippo signaling pathways may lead to new therapeutics for metastasis.

Epidermal Cells

T-1122

RUNX1 AND P21 SYNERGISTICALLY LIMIT THE EXTENT OF HAIR FOLLICLE STEM CELL QUIESCENCE IN VIVO

Jayhun Lee[1], Charelene S.L. Hoi[2], Karin C. Lilja[3], Brian S. White[4], Song Eun Lee[1], David Shalloway[1], Tudorita Tumbar[1]
[1]Molecular Biology & Genetics, Cornell University, Ithaca, NY, United States, [2]Ben May Department of Cancer Research, University of Chicago, Chicago, IL, United States, [3]Sackler Institute of Graduate Biomedical Sciences, New York University, New York, NY, United States, [4]Department of Internal Medicine, Washington University, St. Louis, MO, United States

Mechanisms of tissue stem cell (SC) quiescence control are important for normal homeostasis and for preventing cancer. Cyclin-Dependent Kinase inhibitors (CDKis) are known inhibitors of cell cycle progression. We document CDKis expression in vivo during hair follicle stem cells (HFSCs) homeostasis and find p21, p57 and p15 up-regulated at quiescence onset. P21 appears important for HFSC timely onset of quiescence. Conversely, we find that Runx1, which is known for promoting HFSC proliferation, represses p21, p27, p57 and p15 transcription in HFSC in vivo. Intriguingly, in cell culture, tumors, and normal homeostasis Runx1 and p21 interplay modulates proliferation in opposing directions under the different conditions. Unexpec-

tedly, Runx1 and p21 synergistically limit the extent of HFSC quiescence in vivo, which antagonizes the role of p21 as a cell cycle inhibitor. Importantly, we find in cultured keratinocytes that Runx1 and p21 bind to the p15 promoter and synergistically repress p15 mRNA transcription, thereby restraining cell cycle arrest. This documents a surprising ability of a CDKi (p21) to act as a direct transcriptional repressor of another CDKi (p15). We unveil a robust in vivo mechanism that enforces quiescence of HFSCs, and a context-dependent role of a CDKi (p21) to limit quiescence of SCs, potentially by directly down-regulating mRNA levels of (an)other CDKi(s).

T-1123

IDENTIFICATION OF NOVEL MECHANISMS INVOLVED IN STEM CELL ACTIVATION IN THE SKIN

Jill M. Goldstein, Andrew Chun, Valerie Horsley
Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT, United States

The cellular and molecular mechanisms that regulate hair follicle stem cell activity are not fully understood. We have previously identified that the calcineurin/NFAT pathway controls follicular stem cell activity and inhibition of this pathway can promote hair growth. To identify novel molecular mechanisms that regulate stem cell activity in the hair follicle, we have defined a unique molecular program that is altered in hair follicle stem cells using pharmacological inhibition of calcineurin signaling with cyclosporine A (CSA). This program is distinct from the gene signature of activated stem cells in the hair follicle germ and that induced by CSA treatment in basal keratinocytes of the interfollicular epidermis, suggesting that calcineurin signaling regulates distinct genes in hair follicle stem cells. Specifically, our results identify prolactin receptor (PRLR) as a calcineurin/NFATc1 target gene in follicular stem cells. Inhibition of calcineurin with CSA or genetic ablation of NFATc1 in the skin of mice (NFATc1 cKO) results in the loss of prolactin receptor mRNA and protein expression in hair follicle stem cells. Furthermore, we demonstrate that NFATc1 cKO mice display precocious hair growth during pregnancy, when systemic levels of prolactin are elevated and maintain follicles in a resting state. These findings uncover an NFATc1-PRLR axis that controls stem cell activity in the skin and may reveal the molecular events that contribute to human hair loss associated with pregnancy during the scalp disorder telogen effluvium.

T-1124

NEW INTRINSIC LAYER OF STEM CELLS HOMEOSTASIS REGULATION REVEALED BY COMPETITIVE BALANCE OF INTRABULGE BMP/WNT SIGNALING DURING HAIR CYCLIC ACTIVATION

Eve Kandyba[1], Yvonne Leung[1], Yi-Bu Chen[2], Randall Widelitz[3], Cheng-Ming Chuong[3], **Krzysztof Kobielak**[1]
[A]The Broad CIRM Center for Regenerative Medicine and Stem Cell Research, Department of Pathology, [B]Norris Medical Library, [C]Department of Pathology, [1]University of Southern California, Los Angeles, CA, United States

Hair follicles facilitate the study of stem cells (SCs) behavior because SCs in progressive activation stages are ordered within the follicle architecture and they are capable of cyclic regeneration. We revealed how the underlying molecular mechanisms of Bone morphogenetic protein (BMP) signaling governs the homeostasis of hair follicle stem cells (hfSCs) in vivo. In our recently published model we demonstrated that BMP-inactivated hfSCs exhibit molecular profiles resembling those of hair germs, but still retain multipotentiality after transplantation in vivo. In addition, BMP-inhibited hfSCs display altered BMP and Wnt pathways, exhibiting up-regulation of Wnt7a, Wnt7b ligands and only one Wnt receptor Frizzled (Fzd) 10. We demonstrated that Wnt7a works intrinsically to regulate ligand–receptor-dependent cross talk between BMP and Wnt signaling in hfSCs homeostasis. These results highlight a previously unknown intra-stem cell antagonistic competition, between BMP and Wnt signaling, to balance stem cell activity. We developed this story further and demonstrated the role of Wnt7b in hair follicle Stem Cells regulation, hair morphogenesis and postnatal cycling. Moreover, we also built upon the significance of downstream effectors of canonical BMP signaling, Smads 1/5/8 during hair morphogenesis and postnatal hfSC homeostasis. Collectively, these findings support our discoveries and the existence of one more hierarchical layer regulating stem cell homeostasis in addition to the stem cell–dermal papilla and the hair follicle–adipocyte interaction layers.

T-1125

GENERATION OF FOLLICULOGENIC HUMAN EPITHELIAL STEM CELLS FROM INDUCED PLURIPOTENT STEM CELLS

Ruifeng Yang[1], Ying Zheng[2], George Cotsarelis[2], Xiaowei Xu[1]
[A]Department of lab med and pathology, [B]Department of dermatology, [1]University of Pennsylvania, Philadelphia, PA, United States

Directed differentiation of pluripotent stem cells into a variety of cell types opens new possibilities for regenerative medicine. Epithelial stem cells (EpSCs) in the hair follicle bulge are required for hair follicle growth and cycling, while also contributing to wound healing. Human EpSCs (hEpSCs) in the hair follicle express CD200, ITGA6 and cytokeratin-15 (KRT15) but their isolation and propagation for tissue engineering purposes remains a challenge. Generating sufficient numbers of EpSCs is crucial for treatment of hair loss and other degenerative skin diseases. Here we develop a strategy to differentiate human induced pluripotent stem cells (hiPSCs) into CD200+/ITGA6+ EpSCs that can reconstitute the epithelial components of hair follicles and interfollicular epidermis. CD200+/ITGA6+ EpSCs emerged at day 11 and reached a maximum level around day 18 during the hiPSC differentiation. The hiPSC-derived CD200+/ITGA6+ cells have high colony forming efficiency and show a similar gene expression signature as that of EpSCs isolated directly from human hair follicles. In a skin reconstitution assay, hiPSC-derived CD200+/ITGA6+ cells combined with neonatal mouse dermal cells injected into an immunodeficient mouse produce all hair follicle lineages including hair shaft, inner and outer root sheaths. The regenerated hair follicles consist of a KRT15+ stem cell population and in situ hybridization confirms that the follicular epithelium and interfollicular epidermis are composed entirely of human keratinocytes. Our results indicate that hiPSC-derived CD200+/ITGA6+ cells are molecularly and functionally similar to human hair follicle-derived EpSCs. These results suggest that a sufficient number of folliculogenic human EpSCs can be generated from hiPSCs to develop treatments for hair loss, wounds and other degenerative skin disorders.

T-1126

EPIDERMAL STEM CELL SPRAY FOR BURN WOUND HEALING

Roger Esteban-Vives[1], Matthew T. Young[1], Pattrick Over[1], Peter Rubin[2], Joerg Gerlach[1]
[A]Surgery, [B]Plastic Surgery, [1]Pittsburgh University, Pittsburgh, PA, United States

Introduction
The current treatment for burn wound healing is based mainly on auto-transplantation (Tx). Problems with healing include patient's donor skin area availability, sometimes scar formation, a loss of functionality, and poor aesthetic results. Because of donor area limitations, this method is often insufficient to heal large burn areas, especially larger third degree burn injuries.
Our group developed a therapy using autologous progenitor epidermal cell isolation addressing second degree burn wounds. Previous results from the team suggested that fresh isolated autologous adult dermal fibroblast and allogeneic fetal derived dermal fibroblast contains a MSC-like population. This MSC-like cell population in combination with fibroblast are of interest for regenerative cell-based therapy in full thickness burn healing.
Materials and Methods
Adult epidermal cells were isolated from adult skin biopsies obtained from patients. Adult and Fetal dermal cells were derived from plastic surgery and fetal tissue donations (after medically indicated abortions), respectively. Conceptual in vitro work on dermal cell isolation, cell culture, cell characterization (Flow cytometry and Gene expression), and cell spraying were performed as key proof of concept studies for full thickness therapy. Cell banking work included the development of clones and cryopreservation methods.
Results
Flow cytometry analysis revealed a heterogeneous differentiation stage keratinocyte population including a Stem Cell population (K15+/α6-integrin+) during epidermal cell isolation process. The results of autologous keratinocyte isolation allow us to treat patients in a therapy that is combined with cell spraying for second degree burn healing.

The fetal and adult dermal cell isolation procedure showed a fibroblast population with an additional BM-MSC population characterized by flow cytometry and gene expression. Fetal and adult dermal cells exhibiting also are capable of differentiating to chondrogenic, osteogenic and adipogenic cells using in-vitro assays.
In vitro cultured fetal dermal derived cells revealed BM-MSC-like cell characteristics allowing them as an off-the-shelf product for large burned patients.
Conclusions
As a result of our work, freshly isolated epidermal cells, in combination with spray-Tx, can enhance wound recovery skin regeneration.
Current cell-spray grafting therapy is limited to partial-thickness second degree burn wounds, however, promising preliminary results on dermal cell isolation and in vitro cultured fetal cells (as a cell-banking product), suggest that the therapy can be addressed to third degree burn wounds.

T-1127

CELLULAR AND SIGNALING MECHANISMS THAT REGULATE HAIR FOLLICLE STEM CELLS BY LIVE IMAGING

Valentina Greco
Yale Medical School, New Haven, CT, United States

Stem cells and their niches are critical for tissue development and regeneration. Yet, we still lack knowledge of the sequential steps stem cells undertake to sustain regeneration. Outstanding unanswered questions remain 1) what is the behavior of stem cells, 2) which signals control stem cell behaviors and 3) what is the functional role of the niche during physiological regeneration. A current challenge in the field is having access to a well-defined stem cell niche in which the orderly development of stem cells can be observed, characterized and manipulated in vivo.
My laboratory has recently established an in vivo strategy to visualize the components of the hair follicle stem cell niche, track them over time and manipulate them by two-photon microscopy in live mice. By these means, we have uncovered that hair follicle growth relies on both spatial organization of cell divisions as well as directional cell movements within the stem cell/progeny compartments. In order to address which cells and which behaviors within the stem cell/progeny compartments are controlled by hair follicle regenerative signaling pathways, we have utilized genetic approaches to stabilize the Wnt pathway in subset of cells in combination with our live imaging set-up. Our data suggest that Wnt can control specific stem cell/progeny behaviors depending on the position of these cells within their compartments. In order to dissect whether Wnt effect on specific subset of cells relies on the presence of the niche, we have set up a two-photon based specific cell ablation approach. By these means, we have been able to demonstrate that the niche is required for hair follicle tissue regeneration. We are currently applying these approaches to address whether the Wnt dependent cell behaviors we have identified depend on the presence of the niche.
Thus, we have established an in vivo approach that has led to the discovery of unpredicted cellular mechanisms of growth regulation, and enabled us to precisely investigate functional requirements of stem cell niche components along with key signaling pathways during the process of physiological regeneration.
Acknowledgment: this work is supported in part by NIH-NIAMS and ACS.

T-1128

TCF3 PROMOTES CELL MIGRATION AND WOUND REPAIR THROUGH REGULATION OF LIPOCALIN2

Amy T. Ku[1], Qi Miao[2], Yudai Nishino[2], Jeffrey M. Howard[1], Gloria E. Garcia[2], Ajay S. Rao[2], Diep N. Le[2], Timothy M. Shaver[2], Hoang Nguyen[2]
[A]Interdepartmental program in Translational Biology and Molecular Medicine, [B]Stem Cells and Regenerative Medicine Center, [1]Baylor College of Medicine, Houston, TX, United States

Cell migration is an integral part of re-epithelialization during the skin wound healing, a complex process involving molecular controls that are still largely unknown. Here we identify a novel role for Tcf3, an essential transcription

factor regulating embryonic and adult skin stem cell functions, as a key downstream effector of epidermal wound repair. We show that Tcf3 is upregulated in skin wounds and demonstrate that Tcf3 overexpression accelerates keratinocyte migration and skin wound healing. Promoter analysis and binding studies reveal Stat3 as upstream regulator of Tcf3, which in turn rescues defective cell migration in Stat3-null cells. Mechanistically, we show that pro-migration effects are non-cell autonomous and occur independent of Tcf3/β-catenin interactions of Wnt signaling. Finally, we identify Lipocalin-2 downstream of Tcf3 as the key secreted factor that promotes cell migration in vitro and wound healing in vivo. Our findings provide new insights into the molecular controls of wound-associated cell migration and identify potential therapeutic targets for the treatment of defective wound repair.

Epithelial Cells (Not Skin)

T-1131

DIRECT EVIDENCE BY IN VIVO CLONAL ANALYSIS THAT PROXIMAL TUBULE EPITHELIA DEDIFFERENTIATE TO MEDIATE REPAIR AFTER KIDNEY INJURY

Tetsuro Kusaba, Matthew Lalli, Akio Kobayashi, Benjamin D. Humphreys
Renal Division, Brigam and Women's Hospital, Boston, MA, United States

New treatments for acute kidney injury require a detailed understanding of the mechanisms of proximal tubule repair after injury but a role for intratubular progenitors remains controversial. To define the proliferative potential of differentiated epithelia, we generated a knockin mouse line expressing CreERt2 recombinase in the proximal tubule-specific sodium dependent inorganic phosphate transporter SLC34a1 locus (SLC34a1-CE). After crossing with a reporter line, tamoxifen-dependent recombination was strictly limited to the proximal tubule, with 100% efficiency in the S1 and S2 segments, and up to 65% efficiency in the S3 segment. In maximally labeled nephrons, there was no dilution of genetic label 14 days after injury, showing that intratubular, differentiated cells are wholly responsible for repair without contribution from any other cell source. To assess proliferative potential of individual, we performed clonal analysis of single differentiated tubule cells, after one cycle of ischemia-reperfusion injury and repair. During injury, all labeled cells in the inner cortex expressed the injury marker Kidney Injury Molecule (KIM)-1. Severely injured tubules contained genetically labeled epithelial cells that had a flattened, mesenchymal morphology and were positive for both Ki67 and the mesenchymal marker, vimentin. Thus injured proximal tubular cells underwent dedifferentiation and actively proliferated early after injury. Once repair was complete 14 days later, labeled clones were no longer proliferating and no longer expressed either KIM-1 or vimentin. Average clone size in the inner cortex increased from 1.09 + 0.09 to 1.45 + 0.27 ($p < 0.01$) cells per clone with a reduction in the fraction of single cell clones from 85.9 + 14.3 to 52.9 + 17.8 % ($p < 0.005$). The number of clones consisting of 5 or more cells increased from 0 % before injury to 9.12 + 13.3% ($p < 0.05$) after injury, suggesting that certain clones underwent several rounds of cell division during repair. In primary culture, genetically labeled proximal tubule cells also both proliferated and expressed injury and mesenchymal markers, consistent with our in vivo observations. These results indicate that (a) differentiated epithelia undergo proliferative expansion after injury by transient dedifferentiation and not by a stem or progenitor cell-based mechanism, (b) that most cells proliferate only once or twice during repair (c) but that clones are capable of higher rates of division in areas of more severe damage. Strategies aimed at increasing survival of differentiated cells and enhancing their ability to proliferate after injury should be pursued in order to develop new therapies.

T-1132

THE LUMINAL PROGENITOR COMPARTMENT OF THE NORMAL HUMAN MAMMARY GLAND CONSTITUTES A UNIQUE SITE OF TELOMERE DYSFUNCTION

Nagarajan Kannan[1], Nazmul Huda[2], LiRen Tu[2], Radina Droumeva[1], Geraldine Aubert[1], Ryan Brinkman[1], Peter Lansdorp[1], Satoshi Abe[2], Connie Eaves[1], David Gilley[2]
[1]Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, BC, Canada, [2]Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, United States

Telomere dysfunction-associated genomic instability and elevated activity of telomerase are common features of early stage breast cancers and these changes have been reported to appear in normal human mammary cells that have been extensively passaged *in vitro*. However, little is known about telomere length regulation in the normal human mammary gland. To address this question, we isolated 3 subsets of mammary epithelial cells and the associated stromal cells (depleted of hematopoietic cells and endothelial cells) at >95% purity by fluorescent-activated cell sorting of suspensions prepared from normal reduction mammoplasty tissue obtained from 37 women ranging in age from 17-69 years old. Aliquots of these isolates were then assayed for their in vitro clonogenic cell content as well as their telomere length, evidence of telomere-associated DNA damage and expression of telomerase. The 3 mammary epithelial subsets isolated were basal cells (BCs), and 2 subsets of luminal cells, one of which contains all of the luminal progenitor activity (LPs) and one which lacks such cells (LCs). BCs were prospectively isolated on the basis of their high expression of CD49f and low expression of EpCAM and were found to be highly and selectively enriched in cells with clonogenic myoepithelial±luminal differentiation potential *in vitro* (~20% purity). This subset is also known to contain cell able to regenerate complete gland structures *in vivo* in xeno-transplanted immunodeficient mice. The LPs were isolated on the basis also of their positive expression of CD49f but coupled with high levels of expression of EpCAM. They were confirmed to contain clonogenic cells restricted to luminal differentiation at a purity of ~25%. LPs are thus assumed to represent a stage of differentiation that is intermediate between BCs and LCs.

Telomere length measurements were performed on extracts of pooled cells from each subset by Southern blot (13 samples) and Q-PCR (12 samples) and on single cells by Flow-FISH (3 samples). In every case, the LPs, independent of donor age, were found to have characteristic shorter telomeres (~5 kb) than in the BCs (~8 kb). Gene expression analysis identified significantly elevated levels of DNA damage response (DDR) genes such as *MRE11, RAD50, ATM and ATR* in LPs compared to BCs. We also found that telomere repeat-binding factor (TRF)-2 co-localized with DDR proteins, namely NBS1, MRE11, RAD50, γ-H2AX and 53BP1, at the site of the telomeres within >95% of individually examined LP nuclei (~10 telomere dysfunction-induced foci per LP nucleus). In contrast, such foci were rare in BC nuclei (<5%) isolated from the same tissue samples. The persisting high clonogenic activity of the LPs may be partially explained by the elevated telomerase activity also exclusively displayed by these cells. Interestingly, this latter potential protective mechanism declines with age (r^2=0.5, p<0.009). Taken together, these results reveal marked and previously unappreciated differences in the telomere biology of different subsets of primitive normal human mammary cells. The demonstration of chronically dysfunctional telomeres unique to LPs has potentially important implications for normal mammary tissue homeostasis as well as the development of certain breast cancers.

T-1133

DISTINCT CILIARY PHENOTYPES IN POLYCYSTIC KIDNEY DISEASE AND CILIOPATHY INDUCED PLURIPOTENT STEM CELLS

Benjamin S. Freedman[1], Albert Q. Lam[1], Jamie L. Sundsbak[2], Rossella Iatrino[1], Sarah J. Koon[2], Xuefeng Su[1], Jing Zhou[1], Laurence Daheron[3], Peter C. Harris[2], Joseph V. Bonventre[1]

[1]Renal Division and Center for PKD Research, Brigham and Women's Hospital/Harvard Stem Cell Institute, Boston, MA, United States, [2]Nephrology and Hypertension/Translational PKD Center, Mayo Clinic, Rochester, MN, United States, [3]iPS Core Facility, Harvard Stem Cell Institute, Cambridge, MA, United States

Since pluripotent stem cells are epithelial cells, they hold unique promise for investigating epithelial cell biology and disease. The primary cilium, an apical membrane organelle, was recently identified as central to a family of diseases known as the ciliopathies. Polycystic kidney disease (PKD) is a very common, multi-organ ciliopathy in which epithelial cysts and fibrosis replace the normal kidney architecture. To model PKD in human cells, fibroblasts with characterized mutations from three autosomal dominant (ADPKD) and two autosomal recessive (ARPKD) patients were reprogrammed into induced pluripotent stem (iPS) cells, which could differentiate into diverse tissue types. Undifferentiated iPS and embryonic stem cells elaborated primary cilia and expressed endogenous polycystin (PC)-1 and -2. Polycystin expression levels and rates of proliferation, apoptosis, ciliogenesis, and ciliary length appeared similar in PKD iPS cells and healthy controls. However, a defect in PC2 localization to the primary cilium was

found in ADPKD iPS cell lines: in all three patients, PC2 was detectable in cilia at only one-fourth the frequency seen in ARPKD and healthy control lines. Similarly, somatic epithelial cells and hepatoblasts/biliary precursors differentiated from ADPKD iPS cells had ~50 % fewer cilia with detectable PC2, compared to controls, whereas normal PC2 localization was observed at the endoplasmic reticulum and plasma membrane. Single PKD1 mutations were identified in ADPKD patients and iPS cells, suggesting that the defect did not require loss of heterozygosity. Overexpression of wild-type PC1, but not a carboxy-terminal truncation mutant, in cultured mouse kidney cells caused a 3-5 fold increase in endogenous PC2 levels at the cilium. These results suggest that wild-type but not mutant PC1 enhances PC2 trafficking to cilia. To investigate this mechanism in other ciliopathies comprising a spectrum of multi-organ defects alongside PKD, additional fibroblasts were reprogrammed from patients with Bardet-Biedl Syndrome (BBS) or Meckel Syndrome (MKS). In contrast to ADPKD, neither BBS nor MKS iPS cells exhibited reduced PC2 at the cilium. However, cilia from MKS iPS cells from both patients were twice as long as cilia from BBS, PKD, and control iPS cells, and frequently displayed bulbous or curled ends. These results suggest that iPS cells can recapitulate distinct ciliary disease phenotypes, which may underlie differences in the pathophysiology of different ciliopathies. iPS cells can thus be used to inform human epithelial disease mechanisms and potentially guide the development of therapeutics and regenerative approaches.

T-1134

HIGH-RESOLUTION MICROSCOPY REVEALS CELLULAR CROSSTALK IN THE LIMBAL EPITHELIAL STEM CELL NICHE.

Marc A. Dziasko[1], Hannah Armer[2], Hannah Levis[1], Peter Munro[2], Stephen Tuft[3], Julie Daniels[1]
[A]Institute of ophthalmology - Ocular biology and therapeutics division, [B]Institute of ophthalmology - Imaging unit, [C]Moorfields Eye Hospital NHS Foundation Trust, [1]University College London, LONDON, United Kingdom

Purpose: Maintenance of the ocular surface by limbal epithelial stem cells (LESCs) is essential to preserve the corneal transparency required for vision. Recently our group has identified within the limbus (the transition zone between the central cornea and the conjunctiva) a candidate for the LESC niche: the limbal crypts (LCs). The aim of this study was to demonstrate that the LCs are the reservoir for LESCs but also to identify the putative crosstalk between LESCs and stromal cells taking place in this specific area.
Methods: In vitro clonal analysis was performed to assess the growth potential of LC epithelial cells in comparison to epithelial cells from non-crypt rich areas isolated from human corneal-scleral rims. Transmission electron microscopy followed by serial block-face scanning electron microscopy, manual segmentation and 3D reconstruction were used to image and characterize putative LESCs within their niche.
Results: LC epithelial cells were able to generate holoclones in vitro demonstrating their high growth potential and stem cell characteristics. Imaging LCs by transmission electron microscopy revealed a small size and poorly differentiated “stem-like” cell population closely associated with the underlying stromal fibroblast-like cells, suggesting a possible cell-to-cell interaction. These observations were confirmed by serial block face SEM that highlighted, for the first time, a direct contact between putative LESCs and the surrounding stromal fibroblast-like cells.
Conclusion: These observations directly support the emerging concept that cellular crosstalk maintains cell stemness at the corneal limbus as seen in other epithelial stem cell niches.

T-1135

DYSREGULATION OF NICHE SIGNALS PROMOTE SQUAMOUS METAPLASIA OF HUMAN LIMBAL EPITHELIAL STEM CELLS

Supaporn Khramjuntuk, Praewphan Ingrungruanglert, **Nipan Israsena**
Chulalongkorn Univ, Bangkok, Thailand

Stem cell niche, a microenvironment where stem cells reside, is widely believed to provide important stimuli for stem cell maintenance. Perturbation of tissue-specific niche signals can disrupt tissue homeostasis and involved in disease pathogenesis . For corneal epithelium, it is frequently observed that diseases which cause dry eyes could change non-keratinized stratified ocular surface epithelia into keratinized squamouse epithelia. Yet, the molecular

mechanism for this transdifferentiation is largely unknown. The purpose of this study is to investigate the effect of air exposure on limbal epithelial stem cells(LESCs)and niche cells. Our real-time PCR and immunohistochemistry studies demonstrated that when human limbal explants were cultured with air exposure, there was an up-regulation of TGF-β1 and gremlin, a known BMP antagonist, expression in the superficial layer of limbal epithelial. TGF-β promoted epithelial-mesenchymal change of LESCs as demonstrated by up-regulation of N-cadherin,vimentin, fibronectin,and transcription factor that promote EMT such as slug and snail, In contrast, inhibition of BMP signaling by coculture LESC s on limbal stromal cells overxpressing BMP antagonist resulted in ectopic expression of squamous epithelial specific marker, cytokeratin 1 and 10. Chromatin immunoprecipitation studies showed that TGF-β reduced phosphorylated smad-1 binding on p63 and pax6 promoter. Our data suggest that dysregulation of TGF-β/ BMP signaling may play a role in the squamous metaplasia of LESCs.

T-1136

REDUCTION IN MTDNA COPY NUMBER GENERATES CANCER STEM CELLS AND INDUCES EPITHELIAL-MESENCHYMAL TRANSITION AND IN HUMAN MAMMARY EPITHELIAL CELLS.

Manti Guha, Satish Srinivasan, Anna Kashina, Gordon Ruthel, Narayan Avadhani
Animal Biology, University of Pennsylvania, Philadelphia, PA, United States

Mitochondrial DNA (mtDNA) copy number reduction induces
a calcium-calcineurin dependent mitochondria-to-nucleus retrograde signaling pathway that alters nuclear gene expression and culminates in tumorigenic phenotype in a variety of immortalized cell lines. MtDNA copy number reduction induces epithelial to mesenchymal transition (EMT) in human mammary epithelial cells (hMECs) - MCF10A (non-carcinoma) and MCF7 (carcinoma). This signaling in hMECs induces cellular plasticity to a fibroblast-like morphology and loss of contact inhibition, ultimately conferring increased migratory capacity and invasive phenotype. Interestingly, hMECs with reduced mtDNA copy number had a significant population of $CD44^{high}/CD24^{low}$ cells, a characteristic of mammary cancer stem cells. Moreover, mtDNA copy number reduction generates non-adherent floater-cells with self-renewal capacity which remained viable for ≥ 45days in both 2D monolayer and 3D mammosphere cultures. Additionally, mitochondrial stress signaling induces the expression of mesenchymal markers Snail, Slug, Twist, MMP9, fibronectin and vimentin and is accompanied by loss of expression of epithelial markers e-cadherin and ESRP1 indicative of an EMT. Remarkably, these EMT- like changes are reversed by restoring the mtDNA content indicating that mitochondrial stress signaling drives these events. Our findings suggest a paradigm shift in which mitochondrial genome is a regulator of breast cancer stem cells and EMT. Since breast cancer stem cells are primarily responsible for evading conventional therapy, in future pharmacologic manipulation of mtDNA copy number and intervention of the mitochondrial retrograde signaling pathway may be of therapeutic benefit in metastatic breast tumors.

T-1137

GLOBAL GENE EXPRESSION AND CHEMICAL GENOMICS OF NGF INDUCED ACTIVITY IN PC12 CELL DIFFERENTIATION

Chia-Nan Liao[1], Jack Yu-Shih Lin[3], Mu-Hua Chung[4], Akon Higuchi[2], Qing-Dong Ling[4]
[A]Graduate Institute of Systems Biology and Bioinformatics, [B]Department of Chemical & Materials Engineering, [1]National Central University, Chungli, Taiwan, [2]Department of Ophthalmology, Taipei Medical University Municipal Wan-Fang Hospital, Taipei, Taiwan, [3]Cathay Medical Research Institute, Cathay General Hospital, New Taipei City, Taiwan

The roles of nerve growth factor (NGF) in the treatment of various ocular diseases have been widely investigated both in vitro and in vivo, with growing evidence of associated NGF-pathway alterations. NGF was known to be involved in the modulation of the homeostasis of the ocular surface, as well as the retina and optic nerve. These preliminary clinical reports led us to investigate, through high throughput experiments and system biology analys-

is, the genes and pathways associated with NGF induced neuronal differentiation in PC-12 cells. In this study, after cluster analysis and heat map generation, we identified 2020 NGF-induced genes with altered expression over time. Cross-matching with the KEGG database revealed 830 genes; among which, 395 altered genes were found to have 2-fold increase in gene expression over a two hour period. We then identified 191 associated biologic pathways in the KEGG database; the top 15 pathways showed correlation with neural differentiation. These include the neurotrophin pathways, MAPK pathways, genes associated with axonal guidance and the Wnt pathways. In conclusion, we have established a model system that allows one to systematically characterize the functional pathway changes in a group of neuronal population after an external stimulus. In the present study, we found that after NGF administration in PC12 cells, there is an increase in the number of pathways associated with neuronal differentiation and proliferations. The activation of these pathways may be part of the therapeutic mechanism behind which, NGF administration, either alone or combined with other growth factors, is able to offer a protective role for retinal ganglion cells from degenerative events.

T-1138

α-E-CATENIN CONTROLS DENTAL CELL PROLIFERATION AND DIFFERENTIATION IN ODONTOGENESIS

Chun-Ying Li, Ophir D. Klein
Orofacial Sciences, UCSF, San Francisco, CA, United States

The presence of adult stem cells makes mouse incisors unique compared with molars, because incisors are able to grow continuously throughout the life of the animal. During early tooth development, oral epithelial cells proliferate, invaginate into the underlying mesenchyme, and differentiate into various cell types at later stage. During this process, it is thought that some of the proliferating cells maintain their embryonic characters and become adult stem cells after the incisors are fully formed. It is still unknown how the formation of different cell types, including adult stem cells, is regulated during tooth formation. Here, we show that ablation of α-E-catenin in murine tooth epithelium using K14-Cre induces arrest of tooth bud invagination. Mutant dental epithelium showed abnormal cell polarity at the leading edge, disoriented mitotic spindles, and abnormalities in cell differentiation in the basal layer. The surrounding dental mesenchyme exhibited abnormal condensation and had decreased proliferation. *In situ* hybridization showed that the expression of *Pax9*, *Bmp4* and *Shh*, factors critical for proper tooth development, was decreased. Collectively, these data indicate that α-E-catenin is required for proper cell proliferation and differentiation during formation of the tooth germ. Our findings support a model in which α-E-catenin regulates differentiation of progenitor cells into different cell types in the basal layer of epithelium, and these epithelial cells secrete signaling molecules that regulate dental mesenchyme development.

Embryonic Stem Cell Clinical Application

T-1142

TOWARDS A MACACA FASCICULARIS MODEL FOR AN AUTOLOGOUS TRANSPLANTATION OF IPSC-DERIVED HEMATOPOIETIC CELLS.

Leïla Maouche-Chrétien[1], Soumeya Abed[1], Alisa Tubsuwan[2], Bokojang Chaichompoo[1], In Hyun Park[3], Aïssa Benyoucef[4], Marine Granger-Locatelli[1], Françoise Pflumio[4], Stéphane Prost[1], Emmanuel Payen[1], Geaorge Q Daley[5], Stany Chrétien[1], Philippe Leboulch[1]
[1]INSERM/CEA/iMETI, Fontenay Aux Roses, France, [2]Mahidol university, Bangkok, Thailand, [3]Yale University, New Haven, CT, United States, [4]INSERM/CEA/iRCM, Fontenay Aux Roses, France, [5]HHMI/Children's Hospital Boston/Harvard Medical School, Boston, MA, United States

Induced pluripotent stem cells (iPSCs) are an invaluable source of material for stem cell-based therapies. However, their capacity to give rise to any functional cell type and their therapeutic potential in vivo have not yet been demonstrated. For practical and financial reasons, most in vivo studies are performed in mice and not in large an-

imals, although monkeys are much more closely related to human. Indeed, validation of iPSCs-derived cell-based therapy in simian models is an essential step before iPSCs can be used in humans for regenerative medicine. To advance in this exciting field, we generated iPSCs from primary fibroblasts and bone marrow mesenchymal stem cells of two Macaca fascicularis (cynomolgus) babies. We used RT-PCR, flow cytometry and immunofluorescence assays to confirm the expression of the pluripotent endogenous genes OCT3/4, NANOG, SOX2, REX-1, KLF4, Myc and SSEA4. Intramuscular injection of cy-iPSCs into immunocompromized mice induced the development of teratomas containing tissues of the three germ layers, validating the pluripotency of the cy-iPSCs.
Our aim is to perform autologous transplantation with cy-iPSCs-derived hematopoietic cells into the monkey donor after myeloablation. As a first step, we worked on the optimization of hematopoietic differentiation protocols using co-cultures and embryoid body strategies in presence of three cocktails of hematopoietic cytokines. One protocol was chosen and a hematopoietic differentiation kinetic was followed over 3 weeks by time-lapse FACS analysis and colony forming cells (CFCs) assays to identify emerging cells with hemangioblastic, hematopoietic and endothelial phenotypes with or without CFCs capacity. Fewer than 1/200 CD34+ cells were able to form hematopoietic colonies on methylcellulose, whereas most cells positive for both CD34 and CD45 did form colonies. We used RT-qPCR to analyze the expression of genes associated with mesodermal and hemato/endothelial differentiation during development (among which BRACHYURY, CDX-4, VEGF-R2, SCL, GATA-2, RUNX-1 and GATA-1); This study during 3 weeks of differentiation showed that hematopoietic gene expression was consistent with the hematopoietic cell phenotype as determined by FACS and CFC assays. To evaluate the engraftment potential of cy-iPSC-derived hematopoietic cells, we injected various cell populations (CD34+, CD45+, CD34+ CD45+, total fraction of SSE4- cells) into the femur of sublethally irradiated NSG mice. Among the 30 mice treated, none developed teratoma and seven mice showed engraftment 5 weeks after the injection of Day 20 or a mixture of Days 14 and 18 of differentiated cells. The engraftment was quite low and transient and involved CD34+, CD20+ and CD14+ cells. The species barrier and the origin of the cells could explain this weak result. Large-scale hematopoietic differentiation is now in progress to allow transplantation of CD34+, CD34+CD45+ sorted cells, and 12 differently aged SSEA4- cell fractions, into donor monkeys, and thereby evaluate their capacity for short or long-term hematopoietic engraftment to bring a proof of concept for iPSC-based therapies.

T-1143

CLASSICAL BROWN ADIPOCYTES GENERATED FROM HUMAN PLURIPOTENT STEM CELLS: TOWARDS THERAPUETIC DEVELOPMENT FOR THE TREATMENT OF METABOLIC DISORDERS

Miwako Nishio[1], Masako Nakahara[1], Koichi Saeki[2], Mamoru Hasegawa[2], Akira Yuo[1], Kumiko Saeki[1]

[1]Department of Disease Control, Research Institute, National Center for Global Health & Medicine, Tokyo, Japan, [2]DNAVEC Corporation, Ibaraki, Japan

Brown adipose tissue (BAT) is recognized as a new therapeutic target of obesity and obesity-related metabolic disorders. However, it is hardly possible to obtain enough amounts of functional human BAT samples from living individuals because of ethical and technical hurdles. For stable provision of a sufficient volume of functional human brown adipocytes (BA), an establishment of a method for a direct differentiation of human pluripotent stem cells (hPSCs) into functional classical BA is of great use.
We recently established a method to produce high-purity classical BA from hPSCs including human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC) (Nishio M. et al., Cell Metabolism 16: 394-406, 2012, International Patent pending). At the ISSCR in 2012, we reported that hPSC-derived BA exerted blood glucose level-lowering effects in short-term (~ 24h) transplantation assays. Here we examined longer-term (~ 30 days) effects of hPSC-derived BA on glucose metabolism. Immunocompromised NOG mice, which lack entire lymphocytes, were subcutaneously transplanted with hESC-derived BA and fasting blood glucose levels were measured at Day 10, Day 21 and Day 30. Although we observed statistically significant reductions of fasting blood glucose values in hESC-derived BA-transplanted mice at Day 10 and Day 21, a reduced fasting blood glucose level was detected only in one mouse out of three at Day 30. Thus, subcutaneous transplantation is not suited for longer-term (> 1 month) transplantation experiments. Recently, it was reported that peritoneal transplantation, but not subcutaneous trans-

plantation, of murine BAT exerted long-term (12 ~ 16 weeks) glucose metabolism-improving effects. We are currently studying long-term effects of peritoneally transplanted hPSC-derived BAs on lipid and glucose metabolisms. Our system is based on a completely feeder-free culture and the only xenogenic material used in our method are bovine serum albumin, which can be substituted by recombinant human albumin, and porcine gelatin, which has already been used for clinical purposes. By up-grading the current system to meet with the good manufacturing practice (GMP) grade, we are working toward an establishment of a protocol of cell therapy for metabolic disorders using hiPSC-derived BAs. Also, we are searching for novel human BA-derived adipokine(s) as a potential therapeutic agent for metabolic syndrome using hESC/hiPSC-derived BAs as research tools. Finally, we are studying the transcriptional network that regulates human BA differentiation by applying a Predictive MicroArray Semi-Kinetic (PMASK) analytic system to discover a novel molecular target of drug discovery for the treatment of metabolic syndrome. We will show our recent results on those issues.

T-1145

LONG TERM SELF RENEWAL OF HUMAN PLURIPOTENT STEM CELLS UNDER HUMAN PLACENTA DERIVED CELLS CONDITIONED MEDIUM WITHOUT BASIC FIBROBLAST GROWTH FACTOR SUPPLEMENTATION

Ji Hye Jung[1], Seung Jin Lee[1], Ji Hye Kim[1], Yong Park[2], Byung Soo Kim[2]
[A]Department of Biomedical and science, [B]Department of Internal Medicine, Korea University Medical Center, Seoul, Korea., [1]Institute of stem cell research, Korea University, seoul, Korea, Republic of

Human embryonic stem cells (hESCs) and Human induced pluripotent stem cells (iPSCs) have emerged as a promising source for regenerative medicine. Establishment of an animal substance-free and feeder cell-free environment that supports the self-renewal of human pluripotent cells has been a major goal in the field since hESCs were first derived on fetal mouse fibroblasts. The presence of animal proteins, feeder cells in human pluripotent cell cultures can cause problems related to immunogenicity, microbial and viral contamination and variability of experimental results.
However, many researchers still widely use complex mixture of animal proteins and undefined human proteins that prohibitively expensive for make dose conditions. Previously we reported that feeder formed from human placenta derived cells support prolonged undifferentiated propagation of hESCs without basic fibroblast growth factor (bFGF) supplementation. In this study, we evaluate human placenta derived feeder cells conditioned medium (PCCM) that, with gelatin as an extracellular matrix, allows the long-term culture of hESCs and iPSCs without bFGF. The hESCs and iPSCs self-renewed for at least 6 months (26 passages), after which the cells could *in vitro* differentiation containing cell lineages of all three germ layers. Furthermore, maintaining cellular homogeneity with OCT4, SSEA-4, TRA-1-60, TRA-1-81 positive cells. Using human cytokine antibody array and ELISA, that human placenta derived feeder cells secreted higher amounts of the GRO-a, IL-8, MCP-1, TIMP-2 than the control. Our findings demonstrate, for the first time, identifying novel factors need for hESCs and iPSCs long-term self-renewal and maintenance except for bFGF. This nearly xeno-free and feeder cell-free culture system may be useful for the both hESCs and iPSCs based cell therapies for clinical applications.

T-1146

SEQUENCE-BASED BLOOD-HISTOTYPING RESULT OF A TAIWANESE HUMAN EMBRYONIC STEM CELL COLLECTION

Wei-Le Wang[1], Daphne Kan[4], Tsung-Lin Yang[5], Shao-Yin Chen[6], Kuo-Liang Yang[7], En-Hui Cheng[8], Hsin-Fu Chen[1], Shiaw-Min Huang[6], Chung-Liang Chien[2], Shu-Wha Lin[3], Wannhsin Chen[9], Hong-Nerng Ho[4], **You-Tzung Chen**[1]
[A]Institute of Medical Genomics & Proteomics, [B]Graduate Institute of Anatomy and Cell Biology, [C]Department of Clinical Laboratory Science and Medical Biotechnology, [1]National Taiwan Univ College of Medicine, Taipei, Taiwan, [A]Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, [B]Department of Otolaryngology, [2]National Taiwan Univ College of Medicine and Hospital, Taipei, Taiwan, [3]Bioresources Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan, [4]Tzu-Chi Stem

Cells Center, Tzu-Chi Univ, Hualien, Taiwan, [5]Division of Infertility Clinic, Lee Women's Hospital, Taichung, Taiwan, [6]Biomedical Engineering Research Laboratories, Industrial Technology Research Institute, Hsinchu, Taiwan

Human embryonic stem cells (hESCs) provide a potentially unlimited source for various cell therapies because of their indefinite propagation in vitro and their pluripotent differentiation potential. However histocompatibility is the first obstacle encountered in their regenerative medicine applications. Therefore detailed characterization of the blood-histotypes of the human ES cells is vital to the construction of a hESC bank. Here we report the high-resolution sequence based typing (SBT) of HLA-A,-B,-C,-DQ and -DR loci and acquire complete typing results of eight hESC lines in Taiwan. In our study, there are several hESC lines predicted serologically homozygous at single or double HLA loci: one is homozygous for HLA-A, three are homozygous for HLA-C; one is homozygous for both HLA-C and HLA-DQ loci and one is homozygous for both HLA-A and HLA-C loci. Three of these hESC lines are A blood type (A/A or A/O), three are type O and two are type B (B/B). These documented blood-histotypes of human embryonic stem cell lines as a whole provide detailed reference information for their future use in cell transplantation. The common Asian-specific haplotypes identified and their reported associations with hyper-sensitivity to medications also provide hints for the potential to build drug testing platforms using differentiated tissues derived from these banked human ES cells.

Epithelial Cells (Not Skin)

T-1148

WNT SIGNALING PATHWAY REGULATES THE DIFFERENTIATION OF HUMAN LIMBAL STEM CELLS VIA THE FRIZZLED 7 RECEPTOR

Sophie X. Deng, Hua Mei
UCLA, Los Angeles, CA, United States

Wnt signaling pathway plays an essential role in regulating epithelial stem cell maintenance and differentiation. The possible function of Frizzled (Fz), the co-receptor of Wnt ligand, in regulating limbal stem cells (LSCs) is investigated. Ten Fz members were screened and four Fz receptors (Fz1, Fz4, Fz7 and Fz10) showed consistently higher expressions in the limbus than in the cornea through quantitative real-time PCR. Immunohistochemistry revealed that Fz7 but not Fzq and FZ4 was predominantly expressed in the basal layer of limbal epithelium. The expression of Fz7 was co-localized with the putative stem cell markers including p63α, N-cadherin and K14 and was mutually exclusive to the expression of cornea epithelial maturation marker K12. The expression of Fz7 was higher in the SSEA4- limbal epithelial cells that contain a larger portion of the stem cell population than in the SSEA4+ population. After expansion of the LSCs on 3T3 feeder, the expression level of putative stem cell markers, ABCG2, ΔNp63 and N-cadherin decreased by 77%, 44%, and 73%, respectively (all $p<0.0001$). Interestingly, the expression of Fz7 was also significantly decreased by 82% ($p<0.0001$). After the Fz7 expression was knocked down using shRNA in primary human limbal epithelial cells the expression of ABCG2, ΔNp63α and K14 was decreased by 25%, 20%, and 20%, respectively (all $p<0.05$) compared to the control while the expression of N-cadherin and K12 did not differ from the control population. The colony forming efficiency from the Fz7KD cells was 63% and 38% lower in passage 1 and 2, respectively compared to the control. The findings suggest that Fz7 may be a niche factor of limbal stem cells. Wnt signaling might maintain the undifferentiated state of limbal stem/progenitor cells via the Fz7 receptor.

Hematopoietic Cells

T-1151

TIME DEPENDENT EFFECT OF THE COMBINATION THERAPY WITH ERYTHROPOIETIN AND GRANULOCYTE COLONY STIMULATING FACTOR IN HYPOXIC ISCHEMIC BRAIN INJURY

jihea yu, junghwa seo, jiyong lee, yanghyun cho, sungrae cho

Department & Research Institute of Rehabilitation Medicine, Yonsei University College of Medicine, seoul, Korea, Republic of

Objective: Erythropoietin (EPO) and granulocyte-colony stimulating factor (G-CSF) are likely to play a broad role in the brain. We investigated the effects of the combination therapy with EPO and G-CSF in hypoxic-ischemic brain injury during acute, subacute, or chronic phase.
Methods: A total of 79 C57BL/6 mice with hypoxic-ischemic brain injury were randomly assigned to acute phase (days 1-5), subacute phase (days 11-15) or chronic phase (days 28-32) group. All of which were treated with G-CSF (250 μg/kg) and EPO (5000 unit/kg) or saline daily for 5 consecutive days. Behavioral assessments and immunohistochemistry for angiogenesis, neurogenesis and astrogliosis were performed with an 8-week follow-up. Hypoxia-inducible factor-1α (HIF-1α) was also measured by western blot analysis.
Results: The combination therapy with EPO and G-CSF in acute phase significantly improved rotarod performance (154.25±16.18 sec), In the cylinder test, the difference (Δ) relative to control group in the percentage of wall contacts with contralateral limb was significantly greater in mice treated with EPO and G-CSF in acute phase (14.50±1.73%) compared to mice treated in subacute phase (5.17±2.24%) and chronic phase (-0.33±3.83%) (p=0.002). In ladder walking test, mice treated in acute phase (1.67±0.33%) and subacute phase (2.0±0.68%) showed a significant reduction in the difference (Δ) relative to control group in the percentage of the slips among total steps with hemiplegic forelimbs compared to mice treated in chronic phase (-0.50±0.50%). The acute phase treatment significantly increased CD31(PECAM-1)$^+$ (p=0.001 in frontal cortex, p<0.001 in striatum) and α-smooth muscle actin (α-SMA)$^+$ (p<0.001 in frontal cortex, p=0.012 in striatum) vessels density in frontal cortex and striatum. The acute phase treatment also increased BrdU$^+$/PSA-NCAM$^+$ neurogenesis in subventricular zone (p<0.001), but decreased astroglial density in striatum (p<0.001). Furthermore, the treatment in acute phase significantly increased the HIF-1α expression in cytosol (p=0.007) and nucleus (p=0.048), whereas the treatment in chronic phase did not change the HIF-1α expression, consistent with behavioral outcomes.
Conclusion: Induction of HIF-1α expression by the combination therapy with EPO and G-CSF synergistically enhances not only behavioral function but also neurogenesis and angiogenesis while decreasing the astroglial response in a time-dependent manner.

T-1152

GENERATION AND CHARACTERIZATION OF MACROPHAGES DERIVED FROM MOUSE UTERINE STEM CELLS IN A FEEDER LAYER-LIKE LONG TERM CULTURE SYSTEM

Kota Hatta, Yuemei Zhang, Zhuo Sun, Richard D. Weisel, Ren-Ke Li
Univ of Toronto, Toronto, ON, Canada

The endometrium is a unique tissue in its ability to regenerate. During menses or labor decidua is shed and lost, however new tissue, including the vasculature, is regenerated. Indeed, the endometrium is a rare and highly active site of non-pathological angiogenesis in the adult body. These properties lead us to hypothesize that the uterus contains stem cells that positively contribute to angiogenesis. We sought to culture and characterize uterine stem-cell derived pro-angiogenic macrophages. Using a mouse model, we performed in vitro studies of uterine cells. Mouse uteri were dissected, enzymatically digested and released cells were cultured in IMDM 10% FBS. As the culture progressed, adherent macrophages rounded and emerged above other cells. These rounded macrophages were loosely adherent and could be isolated by tapping the dish or gentle pipetting. The observations (of rounding immune cells forming from adherent cells) were similar to traditional feeder layer cultures of fibroblast cell lines used to differentiate hematopoietic lineage cells. In our primary cell cultures, we identified CD11b+ macrophages rounding above CD90.2- smooth muscle actin+ cells. Future work will examine the potential of uterine CD90.2-smooth muscle actin+ cells as feeder layer cells supporting hematopoiesis, and the angiogenic potential of rounding CD11b+ macrophages. The work supports the notion that the uterine niche is equipped to independently support part of the tissue regeneration that occurs in normal uterine physiology via local, tissue resident stem cells.

T-1153

EXPRESSION OF EBF2 IN OSTERIX-POSITIVE IMMATURE OSTEOBLASTIC CLELS DEFINES A NICHE FOR HEMATAPOIETIC STEM CELLS

Christoph Hinzen, Ursula Zimber-Strobl, **Matthias Kieslinger**
Institute of Clinical Molecular Biology and Tumor Genetics, Helmholtz Zentrum München, Munich, Germany

Hematopoietic Stem Cells (HSCs) reside in specialised bone marrow (BM) microenvironments, which govern their cell fate. During homeostasis, some HSCs within this niche are kept dormant, preserving long-term self-renewal potential, while others self- renew to replenish the hematopoietic system. The stem cell niche mediates signals inducing quiescence, proliferation or differentiation in HSCs. While HSCs themselves are well defined, the composition of the niche is still unclear. Various cell types like mesenchymal stem cells (MSCs), sinusoidal endothelial cells, osteoblasts and adipocytes have been implicated in homing, mobilisation and maintenance of HSCs.
The transcription factor Ebf2 is expressed in mesenchymal progenitors including mesenchymal stem cells (MSC), immature osteoblastic cells and adipocytes, but not in hematopoietic cells. HSCs localise to Ebf2-expressing cells at the endosteum and deletion of Ebf2 in mice results in an age-dependent loss of hematopoietic cells and decreased frequencies of HSC. Purified Ebf2-expressing cells of the bone marrow demonstrate a need for Ebf2 and cell-cell contact in the support of HSC, clearly implicating Ebf2 in the niche-dependent support of HSC. However, as Ebf2 is expressed by various mesenchymal cell lineages, the individual contribution of these lineages is not clear. To address this question, we have generated Ebf2 conditional knock-out mice, and crossed them with MSC, adipocyte and osteoblast-specific Cre lines. Deletion of Ebf2 specifically in immature osteoblastic cells using the Osx-Cre line fully recapitulates the defect in HSC maintenance of the conventional Ebf2 knock-out, whereas loss of Ebf2 in adipocytes has no detectable consequences on HSC frequency. A DNA microarray analysis of Ebf2-expressing cells reveals genes involved in homing and proliferation of HSCs as down-regulated in Ebf2-deficient cells, partly explaining the observed phenotype. As Ebf2 mediates its activity in HSC maintenance directly via cell-cell contact, we conclude that immature osteoblastic cells constitute a part of the niche for HSC and we will present first data to gain a mechanistic understanding of its supportive capacity.

T-1154

17β-ESTRADIOL HAS A BIPHASIC EFFECT ON THE FORMATION OF HEMATOPOIETIC STEM CELLS

Kelli J. Carroll[1], Michael C. Dovey[1], Claire C. Cutting[2], Maija K. Garnaas[2], Virginie Esain[1], Gregory Frechette[1], Daniel Gorelick[3], James M. Harris[1], Marnie Halpern[3], Wolfram Goessling[2], Trista E. North[1]
[1]Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, MA, United States, [2]Brigham and Women's Hospital/Harvard Medical School, Boston, MA, United States, [3]Carnegie Institute for Science, Baltimore, MD, United States

While intrinsic signaling pathways regulating hematopoietic stem cells (HSC) are increasingly understood, less is known about the consequences of exposure to environmental factors. Through a chemical genetic screen, estrogen-related compounds were identified as modifiers of *runx1* expression in the zebrafish. Exposure to 17beta-estradiol (E2) significantly decreased the number of *runx1+* HSCs in the AGM compared to controls by *in situ* hybridization, FACS, and qPCR. MO-knockdown and use of the nonspecific estrogen receptor inhibitor fulvestrant confirmed that estrogen was required for nascent HSC regulation and functioned through classical estrogen receptors. Using an ERE-GFP reporter and enzyme immunoassay, estrogen activity was detected in the AGM during hematopoiesis, with estrogen receptors *esr1 (esr alpha)* and *esr2a/b (esr beta)* demonstrating differential spatio-temporal expression in vascular and hematopoietic cell types. E2 treatment from 12-24 hours post-fertilization disrupted vessel formation and angiogenic sprouting, as indicated by *flk1* (*kdrl*), and altered the assignment of *ephrinb2+* arterial identity. Consistent with that phenotype, E2 exposure decreased activity in a Notch pathway-reporter line and blocked arterial expression of *deltaC* and *notch5* as well as *VEGFAa* expression, upstream of the notch cascade. Hyperactivation of either the VEGF or Notch pathways could rescue the hematopoietic defects observed following E2 exposure, confirming changes in this transcriptional network underlie the observed phenotypes. To determine whether environmental estrogens could mediate similar effects, we exposed embryos to the phytoestrogen gen-

istein, the synthetic estrogen ethinylestradiol (EE), and the xenoestrogen bisphenol A and found each decreased formation of HSCs; using fulvestrant, we confirmed the effects were at least partially dependent on estrogen receptor stimulation. In addition, EE and genistein had the ability to decrease signaling in Notch:GFP reporter fish. Interestingly, when exposure to E2 occurred after arterial establishment and initiation of blood flow, from 27-34 hpf, estrogen treatment enhanced HSC number by *in situ* and qPCR. Markers of the cell cycle including cyclinD1 and c-myc were significantly increased suggesting estrogen enhances cell cycling. This proliferative effect did not appear to be dependent on Notch or VEGF signaling as inhibition of either pathway could not block the increase in HSCs following E2 exposure. Effects of E2 on primitive hematopoiesis were also noted: E2 treatment enhanced expression of *gata1* both by *in situ* and FACS while repressing the expression of *globin*, suggesting that while estrogen enhances the self-renewal of progenitors, it may impair their differentiation into mature lineages. In an adult zebrafish marrow injury model, E2 significantly accelerated stem and progenitor cell recovery in both sexes. Intriguingly, females, with higher circulating estrogen levels, recovered better after injury than males, both in the presence and absence of exogenous estrogen. Finally, functional conservation of estrogenic effects was confirmed with a murine CFU-S_{12} assay. Together, these data identify estrogen and estrogen-related compounds as critical regulators of hematopoietic stem cell formation and proliferation and suggest a novel role for estrogen in the development of the hematopoietic niche.

T-1155

DNMT3B IS DISPENSABLE FOR HEMATOPOIETIC STEM CELL FUNCTION, BUT ACTS SYNERGISTICALLY WITH DNMT3A TO CONTROL THE BALANCE BETWEEN SELF-RENEWAL AND DIFFERENTIATION

Grant A. Challen[1], Allison Mayle[2], Mira Jeong[2], Min Luo[2], Deqiang Sun[2], Benjamin Rodriguez[2], Wei Li[2], Margaret Goodell[2]

[1]*Division of Oncology; Dept of Internal Medicine, Washington University in St. Louis, Saint Louis, MO, United States,* [2]*Baylor College of Medicine, Houston, TX, United States*

DNA methylation of CpG dinucleotides is one of the major epigenetic modifications in the vertebrate genome. DNA methylation is catalyzed by a family of DNA methyltransferase enzymes comprised of Dnmt1, Dnmt3a, and Dnmt3b. In humans, somatic mutations in DNTM3A have been identified in ~20% of human acute myeloid leukemia (AML) and ~10% of myelodysplastic syndrome (MDS) patients, but the mechanisms through which these mutations contribute to pathogenesis is not well understood. Congenital mutations in DNMT3B can cause ICF (immunodeficiency, centromeric instability, and facial anomalies) syndrome. These patients exhibit chromosomal instability due to heterochromatin decondensation and demethylation of satellite DNA.

We have recently shown that Dnmt3a is essential for hematopoietic stem cell (HSC) differentiation. Conditional knockout of Dnmt3a (Dnmt3a-KO) resulted in HSCs that could not sustain peripheral blood generation after serial transplantation, while phenotypically defined HSCs accumulated in the bone marrow. Given Dnmt3b is also highly expressed in HSCs, we reasoned it may also have a specific role in HSC function. Here, we examine the role of Dnmt3b, alone and in combination with Dnmt3a inactivation, in the regulation of hematopoiesis. We performed conditional deletion of Dnmt3b in HSCs, as well as Dnmt3a and Dnmt3b simultaneously, using the Mx1-cre system. Unlike the Dnmt3a-KO HSCs, loss of Dnmt3b had a minimal impact on blood production. Even after several rounds of transplantation, Dnmt3b-KO HSCs performed similarly to controls. However, the Dnmt3ab-dKO (double knockout) peripheral blood contribution was quickly and severely diminished, accompanied by a dramatic accumulation of Dnmt3ab-dKO HSCs in the bone marrow (>50-fold), paralleling the phenotype of the Dnmt3a-KO HSC, but more extreme.

To examine the impact of loss of Dnmt3a and -3b on DNA methylation in HSCs, we performed Whole Genome Bisulfite Sequencing (WGBS) on Dnmt3a-KO, Dnmt3ab-dKO and control HSCs. While loss of Dnmt3a led to both increases and decreases of DNA methylation at distinct genomic regions, ablation of both Dnmt3a and -3b primarily resulted in loss of DNA methylation that was much more extensive than that seen in the absence of Dnmt3a alone. In addition, RNA-SEQ of the mutant HSCs revealed loss of transcriptional integrity including increased expression of repetitive elements, inappropriate splicing, and premature truncation of 3'UTRs.

These data show that Dnmt3a and -3b act synergistically to regulate HSC differentiation. The accumulation of Dnmt3ab-dKO HSCs cannot be attributed to altered proliferation or apoptosis, but rather the lack of peripheral blood contribution is due to an imbalance between self-renewal and differentiation cell fate decisions. By understanding the mechanisms through which Dnmt3a and -3b exert these effects, we should identify genes that are critical for normal hematopoietic differentiation. These genes may serve as targets for therapeutic intervention in malignancies caused by defective DNA methyltransferases. This work highlights the importance of DNA methylation in HSC cell fate decisions and further contributes to understanding the epigenetic regulation of hematopoiesis which is still poorly understood.

T-1156

THE ROLE OF IGFII IN THE REGULATION OF HEMATOPOIETIC STEM CELL FUNCTION

Dolly Thomas, Andreia Gianotti Sommer, Gustavo Mostoslavsky
Medicine, Boston University School of Medicine, Boston, MA, United States

Maintenance of the hematopoietic system is dependent on the proper regulation and function of HSC (hematopoietic stem cell) populations contained within the bone marrow niche. Preliminary microarray data comparing gene expression profiles between LT-HSC (SP Lin- Sca1+ cKit+CD34-), ST-HSC (SP Lin- Sca1+ cKit+CD34+), and non-HSC of the bone marrow have revealed a significantly higher expression of IGFII within LT-HSC when compared to non-HSC populations, suggesting a potential role for IGFII in HSC regulation. In vitro methylcellulose colony forming (CFU) assays containing IGFII overexpressing HSC, have yielded a greater percentage of multi-lineage colonies indicative of the maintenance of HSC self-renewal properties. Utilizing in vivo competitive transplant assays we have shown that IGFII transduced HSC exhibit enhanced long-term donor contribution upon bone marrow transplant. We hypothesize that IGFII regulates HSC self-renewal by affecting cell cycle function, specifically by its effects on p57 expression. P57, a member of the Cip/Kip family of cyclin dependent kinase inhibitors, has recently been shown to be required for regulation of HSC quiescence and long-term self-renewal. Our studies show that lentiviral mediated overexpression of IGFII within purified HSC alters expression of p57, suggesting that an IGFII/p57 pathway is involved in the regulation of HSC function.

T-1157

THE ATM-BID-MTCH2 MITOCHONDRIAL PATHWAY REGULATES HAEMATOPOIETIC STEM CELL FATES

Maria Maryanovich, Atan Gross
Biological Regulation, The Weizmann Institute of Science, Rehovot, Israel

The BH3-only BID protein plays a critical function in the death-receptor pathway in the liver by triggering mitochondrial apoptosis. Interestingly, BID is also involved in cell cycle regulation, and this function is monitored by the ATM DNA damage kinase. Recently we demonstrated that ATM-mediated BID phosphorylation plays an unexpected role in maintaining the quiescence of haematopoietic stem cells (HSCs). Loss of BID phosphorylation leads to escape from quiescence of HSCs, resulting in exhaustion of the HSC pool and a marked reduction of HSC repopulating potential in vivo. We further demonstrated that the regulation of both quiescence and survival of HSCs depends on BID's ability to regulate the levels of reactive oxygen species (ROS) at the mitochondria.
How does BID regulate mitochondrial function/ROS in the haematopoietic system? Recently we demonstrated that mitochondrial carrier homologue 2 (MTCH2), a novel protein suggested to be involved in obesity, functions as a mitochondrial receptor for BID and is essential for Fas-induced apoptosis in vivo. To investigate the role of MTCH2 in the bone marrow, we generated MTCH2 haematopoietic-specific knockout (HKO) mice using the Vav1-Cre mice. HKO mice demonstrate a decrease in long-term (LT) HSCs and expansion of common lymphoid progenitors. Interestingly, MTCH2-deletion also results in an increase in mitochondrial mass in both LT-HSCs and restricted progenitors, suggesting that changes in mitochondria function/metabolism play an important role in regulating cellular fates in the haematopoietic system.

Taken together, our data are consistent with the idea that MTCH2 is a new player in the ATM-BID pathway, and that this novel mitochondrial pathway plays a critical metabolic function in regulating HSC survival and quiescence.

T-1158

CO-INHIBITION OF THE TNF-JNK-AP1 AXIS AND NF-κB SYNERGIZE IN AML STEM AND PROGENITOR CELLS

Andrew Volk[1], Jin Li[2], Junping Xin[1], Dewen You[1], Peter Breslin[1], Wei Wei[1], Rachel Schmidt[1], Sucha Nand[1], Zejuan Li[3], Jianke Zhang[4], Jianjun Chen[3], Jiwang Zhang[1]
[1]Loyola University Chicago, Maywood, IL, United States, [2]Shanghai Normal University, Shanghai, China, [3]University of Chicago, Chicago, IL, United States, [4]Thomas Jefferson University, Philadelphia, PA, United States

Leukemic stem cells (LSCs) isolated from acute myeloid leukemia (AML) patients are more sensitive to NF-κB inhibition-induced cell death *in vitro* when compared to normal hematopoietic stem/progenitor cells (HSPCs). This suggests that blocking NF-κB could be an effective strategy for treating AML. However, the inadequate anti-leukemic activity of NF-κB inhibition *in vivo* suggests that elements within the bone marrow microenvironment might stimulate compensatory survival/proliferation signals in AML cells that can overcome the effects of NF-κB inhibition. Our data suggests Tumor Necrosis Factor-α (TNF) mediates such a signal. We found TNF levels are significantly increased in samples from most AML patients, especially AML subtypes M3, 4 and 5 when compared to samples from healthy donors. We also found that many types of AML cells produce TNF.
Stimulating HSPCs with exogenous TNF induces powerful RIP1/3-mediated necroptosis and caspase-mediated apoptosis responses, resulting in cell death and loss of normal hematopoietic function. TNF-mediated cell death in HSPCs can be partially blocked by inactivation of JNK-AP1 signaling.
In our AML model, stimulation with exogenous TNF promotes the *in vitro* growth of leukemic cells by stimulating both NF-κB and JNK-AP1 signaling. We found that while TNF-JNK-AP1 pathway activation in HSPCs leads to cell death, leukemic cells convert TNF-JNK-AP1 signaling from a death signal to a survival signal. Inactivation of any part of the TNF-JNK-AP1 signaling axis can repress the growth of leukemic cells *in vitro* and delay leukemogenesis *in vivo*. We also show that blocking any portion of the TNF-JNK-AP1 signaling axis sensitizes TNF-expressing leukemic cells, including LSCs, to NF-κB inhibitor treatment while at the same time protecting HSPCs from such treatment.
In conclusion, we found that many types of AML cells produce TNF. This TNF can act in an autocrine fashion to promote LSC survival and self-renewal, as well as in a paracrine fashion to repress normal hematopoiesis. Our studies also suggest that the inadequate ability of NF-κB inhibition to clear AML tumors *in vivo* is due to the compensatory TNF-mediated activation of the JNK-AP1 signaling axis in LSCs. Therefore, we propose that inhibition of both TNF-JNK-AP1 and NF-κB signals may provide a more thorough treatment for AML patients with elevated peripheral blood TNF.

T-1161

EX VIVO EXPANDED HUMAN UMBILICAL CORD BLOOD MYELOID PROGENITORS CELLS STIMULATE VASCULAR REGENERATION

David M. Putman, Ayesh Seneviratne, David A. Hess
Department of Physiology and Pharmacology, University of Western Ontario, London, ON, Canada

Effective therapies to promote revascularization are central to the treatment of ischemic diseases including myocardial infarction, stroke and peripheral vascular disease. However, clinical implementation of cellular therapies is impeded by our limited understanding of the many cell types and signaling effectors implicated in the coordination of vascular repair. Due to recent initiatives to collect and store umbilical cord blood (UCB) for regenerative therapies, UCB-derived progenitors have emerged as a readily-available allogeneic source of pro-angiogenic cells untouched by chronic cardiovascular disease-related pathologies.
We have recently reported that human UCB-derived multipotent myeloid progenitor cells, prospectively purified based on high aldehyde dehydrogenase activity ($ALDH^{hi}$), can promote the recovery of perfusion in immunodefi-

cient mice with acute unilateral hindlimb ischemia. Intravenous transplantation of $2x10^5$ freshly isolated human UCB $ALDH^{hi}$ cells augmented reperfusion and stimulated revascularization without long-term engraftment in the ischemic limb (*Putman et al.* Stem Cells 2012). High ALDH activity enriched for cells that demonstrated increased production of pro-angiogenic cytokines by microarray, and promoted endothelial cell survival and tube formation during co-culture. Unfortunately, the rarity of $ALDH^{hi}$ progenitor cells (0.4±0.1% of total mononuclear cells) impedes clinical implementation of cell-based therapies due to difficulty achieving clinically relevant cell numbers.

To address this issue, we have optimized *ex vivo* culture protocols to increase the number of UCB $ALDH^{hi}$ cells available for therapy. UCB $ALDH^{hi}$ cells were grown on fibronectin-coated plates in serum-free, X-vivo15 media supplemented with recombinant human SCF, Flt-3L, and TPO (10ng/mL). After 6 days of culture, we observed 14.0±5.6 fold expansion in total cell number (n=10), and 13.2±2.7% of cells retained high ALDH-activity, Expanded $ALDH^{hi}$ cells maintained primitive progenitor (77.6±4.2% $CD34^+$, 48.7±7.7% $CD133^+$, 5.46±1.2% $CD117^+$) and early myeloid markers (99.9±0.2%, CD33+), an expression pattern similar to freshly isolated UCB $ALDH^{hi}$ cells (n=5). Furthermore, expanded cells maintained multipotent myeloid colony forming cell function in methylcellulose media (1 CFU in 10 cells). Remarkably, intramuscular injection of these expanded myeloid progeny into mice with acute unilateral ischemic injury stimulated robust vascular recovery with kinetics similar to uncultured UCB $ALDH^{hi}$ cells. Within 7 days post-transplantation, *ex vivo* expanded myeloid progenitors ($5x10^5$ cells) significantly accelerated the recovery of perfusion (RP) in the ischemic limb (RP=40±7%, n=8) compared to saline-injected mice (RP=25±8%, n=15, $p<0.05$), and augmented perfusion was maintained for one month post-transplantation (RP=71±16% vs 39±12%, $p<0.01$). Progenitor cell transplanted mice also showed higher $vWF^+/CD31^+$ capillary density in the ischemic limb compared to saline injected controls ($p<0.01$).

Collectively, these data indicate that UCB $ALDH^{hi}$ cells can be efficiently expanded in serum-free *ex vivo* culture without significant loss of progenitor phenotype or vascular regenerative capacity, and provide proof-of-concept data to support the clinical translation of allogeneic cell-based therapies for ischemic disease.

T-1162

EX VIVO EXPANSION OF HEMATOPOIETIC STEM CELLS CULTURED ON BIOMATERIAL HAVING DIFFERENT ELASTICITY

Jui-Hsiang Hsiao, Yu-Hsuan Chou, Pin-Yu Wang, Akon Higuchi
National Central Univ, Jhongli City, Taiwan

Umbilical cord blood (UCB) is an attractive source of hematopoietic stem and progenitor cells for hematopoietic stem cell (HSC) transplantation. However, the low number of HSCs obtainable from a single donor of UCB limits direct transplantation of UCB to the treatment of pediatric patients. In this study, we investigated the ex vivo expansion of HSCs cultured on biomaterials grafted with several nanosegments, which have different elasticity. Matrix elasticity of cell culture biomaterials plays an important role in maintenance of pluripotency and differentiation abilities of stem cells. Physical properties (e.g., elasticity) of the culture substrates can affect the stem cell fate of differentiation and pluripotency of the stem cells. Similarly, the chemical and biological properties of cell culture substrates can also affect the maintenance of pluripotency and differentiation ability of the stem cells. In this study, we prepared polyvinylalcohol-co-itaconic acid (PVA-IA) coating dishes having different stiffness from 0.022 MPa to 16.5 MPa where fibronectin or oligopeptide derived from cell binding domain of fibronectin (CS1, EILDVPST) were grafted. The elasticity of PVA-IA films were controlled by crosslinking time of PVA-IA films with glutaraldehyde (30 min-24 hrs). HSCs were isolated from UCB by Ficoll-Paque method followed by CD34 selection through magnetic associated cell sorting method (MACS). Ex vivo expansion of HSCs was the highest when HSCs were cultured on stiffer PVA-IA coating dishes without grafting of fibronectin nor CS-1 oligopeptide in HSC expansion medium. The pluripotent colony-forming units (i.e. colony-forming unit-granulocyte, erythroid, macrophage, and megakaryocyte [CFU-GEMM]) from HSCs were also found to increase with increasing the elasticity of PVA-IA coating dishes when HSCs cultured on PVA-IA coating dishes without immobilization of fibronectin and CS-1 were used for the colony-forming assay. On the other hand, when HSCs were cultured on PVA-IA coating dishes grafted with fibronectin or CS-1,

high expansion fold of HSCs was observed on the PVA-IA dishes having intermediate stiffness from 0.12 MPa to 1.5 MPa, which was more than 1.6 times higher than expansion fold of HSCs cultured on conventional tissue culture polystyrene dishes (TCPS, 12 GPa). Furthermore, HSCs cultured on PVA-IA coating dishes grafted with fibronectin or CS-1 having 12.2-30.4 MPa showed more pluripotent colony-forming units (i.e. CFU-GEMM) than those on TCPS dishes (12 GPa). This result indicates that both physical and biological properties of cell culture materials will affect the ex vivo expansion of HSCs. The biological cues of cell culture materials such as fibronectin or CS-1 grafting on the cell culture dishes seem to play a more significant role than the physical characteristics (elasticity) of cell culture materials during ex vivo expansion of HSCs on the 2D cultivation.

T-1163

EFFICIENT EXPANSION OF UMBILICAL CORD BLOOD DERIVED CD34$^+$ CELLS IN A CO CULTURE WITH MESENCHYMAL STEM CELLS IN THE PRESENCE OF APOPTOTIC INHIBITORS

Lalita Limaye, Vaijayanti Kale, Darshana Kadekar
National Centre For Cell Science, Pune, India

Umbilical cord blood (UCB) transplantation is an established procedure for the treatment of hematologic, immune, and genetic diseases mainly in pediatric population. An inadequate number of HSCs present in a single UCB unit however limits its use in adults as it may delay engraftment and consequently lead to higher rates of graft failure. One of the strategies to overcome this limitation is the ex vivo expansion of HSCs. The involvement of apoptosis during expansion is well documented and has been accounted for inferior quality of expanded cells. Earlier we have demonstrated the beneficial effect of the apoptotic inhibitors on expansion of HSCs in suspension cultures. In order to recapitulate the apoptotic signaling operative between the stromal cells and HSCs, in the present work, we have attempted to modify this expansion strategy by regulating the apoptosis in HSC MSC co-culture system.
UCB-derived CD34+ cells were isolated and cultured either in suspension cultures or in co-cultures with MSCs derived from human cord tissue viz, cord (CMSCs) and placenta (PMSCs) for 10 days. The cultures comprised of serum-free medium supplemented with 25ng /ml each of SCF, TPO, Flt-3L and IL-6 in presence or absence of the pan-caspase inhibitor zVADfmk (500nM) or calpain-1 inhibitor zLLYfmk (15uM). Cells were harvested and the level of expansion was analyzed by phenotypic characterization and functional assays.
The cells expanded in the co-cultures in the presence of apoptotic inhibitors displayed higher proliferation with significantly more retention of the primitive CD34$^+$CD38$^-$ and CD 133$^+$ cells as compared to suspension cultures as well as control co-cultures. Apoptotic inhibition during co-cultures with MSCs significantly increased the *in vitro* clonogenicity, migratory and adhesion abilities as compared to the suspension cultures. Amongst the feeders we observed a difference in the ability of the CMSCs vs. the PMSCs for the expansion of HSCs. To rule out sample to sample variation we used PMSCs and CMSCs from the same donor. Though there was no significant difference in the morphology and surface marker expression in CMSCs and PMSCs, we observed that co-culture with the PMSCs in presence of apoptotic inhibitors harbored significantly more primitive HSCs with improved in vitro functionality. Thus we conclude that the synergy between MSCs and apoptotic inhibitors facilitate superior *ex vivo* expansion of HSCs. The placental MSCs in presence of apoptotic inhibitors pose a better system for expansion of UCB CD 34+ cells .Therefore our expansion strategy which results in superior quality graft may find direct application in UCB transplantation settings in the clinics.

T-1164

VITAMIN D3 NEGATIVELY REGULATES HEMATOPOIETIC STEM CELL DEVELOPMENT BY AFFECTING VASCULAR NICHE FORMATION THROUGH INHIBITION OF HEDGEHOG SIGNALING

Mauricio Cortes[1], Kelsey Natsuhara[2], Wolfram Goessling[3], Trista E. North[1]
[1]Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States, [2]Harvard University, Cambridge, MA, United States, [3]Division of Genetics and Gastroenterology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States

Studies of the vitamin D3 signaling pathway have revealed a broad role for this hormone in tissue homeostasis and as a result there is great interest in exploring the therapeutic potential of vitamin D3 for the treatment of various diseases. While in vitro studies have shown that Vitamin D3 exhibits anti-proliferative and pro-differentiation effects on leukemic cell lines, its role in hematopoietic stem cell (HSC) homeostasis and leukemia progression in vivo is not well understood. To elucidate the mechanisms of action of vitamin D3 in vivo during HSC self-renewal and differentiation we turned to the zebrafish as an animal model due to their evolutionary conserved blood system and their amenability for genetic and chemical manipulation. Vitamin D3 was identified in a chemical as a negative regulator of hematopoiesis in the aorta-gonad mesonephros (AGM), the first site of definitive hematopoiesis. Treatment of zebrafish embryos from early somitogenesis to 36hpf with the non-hydroxylated vitamin D3 precursor cholecalciferol (D3) resulted in decreased mRNA expression of the HSC markers runx1 and c-myb as determined by whole mount in situ hybridization (WISH) and by reduced GFP expression in the AGM of Tg(runx1P2:egfp) zebrafish embryos. To quantify the reduction in HSCs observed by WISH, FACS analysis was performed on double positive Tg(Lmo2:dsRed), Tg(c-myb:gfp) embryos revealing a 25% decrease in the number of double positive cells corresponding to HSC progenitors at 36hpf. To elucidate the mechanism of action of D3 on HSC formation, embryos were treated between 12-24hpf (the period of vascular niche formation) and 24-36hpf (the period of HSC induction and expansion). Treatments with D3 after 24hpf did not have an effect in runx1/c-myb expression. In contrast, exposure of embryos with D3 from 12-24hpf resulted in a decrease in runx1 expression at 36hpf suggesting that D3 is acting during the establishment of the vascular niche. Vascular niche formation is a highly orchestrated process regulated by a cascade of signaling pathways including hedgehog (Hh) signaling. Based on the importance of Hh signaling in artery/vein specification and published in vitro data on the role of D3 in Hh pathway inhibition, we postulated that the negative effect of D3 on HSC formation was mediated through decreased in Hh signaling. In support of our hypothesis, treatment with D3 resulted in decreased Hh signaling as determined by a reduction in ptch2 mRNA expression via WISH, and by reduced Gli reporter activity as measured by FACS using the Tg(6xGLi:mCherry) reporter line. Interestingly co-treatments of zebrafish embryos with the Hg antagonist cyclopamine and D3 resulted in severe inhibition of Hh signaling and loss of runx1 positive cells in the AGM revealing synergy between these two compounds. In addition, co-treatment with the Hh agonist SAG rescued the HSC defect in D3 treated embryos. Hh signaling regulates vein/artery specification through the activation of notch. Consistent with reduction in Hh signaling, D3 treated embryos had a reduction in notch reporter activity and expansion of venous cell fate with concomitant reduction in artery specification. In sum, these studies revealed vitamin D3 acts as negative regulator of HSC formation by inhibiting hedgehog-notch signaling and affecting vascular niche specification.

T-1165

THE TRANSCRIPTION FACTOR AND EMT MEDIATOR ZEB2 IS AN ESSENTIAL REGULATOR OF DIFFERENTIATION IN ADULT MURINE HEMATOPOIETIC STEM CELLS

Tamara Riedt[1], Steven Goossens[2], Ines Guetgemann[1], Carmen Carrillo Garcia[1], Hichem Gallala[1], Jin Li[1], Holger Froehlich[1], Peter Brossart[1], Danny Huylebroeck[3], Jody Haigh[2], Viktor Janzen[1]
[1]University of Bonn, Bonn, Germany, [2]University Ghent, Ghent, Belgium, [3]University of Leuven, Leuven, Belgium

The life long replenishment of highly specialized blood cells by a small number of hematopoietic cells (HSC) requires a strict regulation between self-renewal and differentiation in the immature compartment of the bone marrow. Perturbation of this equilibrium can result in hematopoietic failure due to maturation defects or development of hematological malignancies. The regulation of self renewal and differentiation processes are mainly controlled by a network of transcription factors. Zeb2 is a transcriptional repressor that plays an important role during embryonic development as a modulator of the epithelial to mesenchymal transition (EMT) and is also associated with tumor progression and metastasis. We have recently reported thatZeb2 is essential for murine embryonic hematopoiesis. The aim of this study was to define the role of Zeb2 in the regulation of homeostasis in the adult hematopoietic system.
Using an inducible Zeb2 knock out mouse model based on the Mx1-Cre promoter we analyzed the impact of Zeb2 loss on adult hematopoietic stem cell function. Upon Zeb2 deletion mice developed severe cytopenia with drastic

reduction in thrombocytes, monocytes, B- and T- lymphocytes and progressive anemia.. However, analysis of the primitive bone marrow compartment revealed that Zeb2 deletion resulted in a pronounced increase in the most immature hematopoietic cells (Lin-Sca1+cKit+CD48-CD150+). In addition, we observed a perturbation in different lineage restricted progenitor subpopulations upon Zeb2 deletion. No difference in cell cycling activity or apoptotic rate in the stem cell enriched bone marrow population was detectable between the genotypes. Transplantation of Zeb2-deficient bone marrow cells into lethally irradiated wild type recipients revealed significantly reduced ability of Zeb2-deficient stem cells to differentiate into multiple hematopoietic lineages. On the other hand, transplantation of WT cells into a Zeb2-deficient microenvironment displayed no obvious maturation defects. These results indicate a niche-independent effect of Zeb2 in promoting differentiation of hematopoietic stem cells.
In addition, conditional Zeb2 knock out animals displayed several features of a myeloproliferative disease resembling the early stages of human primary myelofibrosis, including predominant myeloid proliferation in the bone marrow accompanied with morphological abnormalities in megakaryocytes and reticular fibrosis. Furthermore, Zeb2-deficient mice also displayed progressive extramedullary hematopoiesis in spleen and lymph nodes.
At the molecular level, Zeb2 deficiency resulted in upregulation of numerous genes, as shown by microarray analysis from isolated primitive hematopoietic cells, consistent with its known role as transcriptional repressor. Among those we identified GATA-2 and Id2, both molecules involved in the differentiation process as putative targets of Zeb2.
Our data thus indicate that Zeb2 is involved in the regulation of differentiation at multiple stages of hematopoietic cell maturation. Furthermore, lack of Zeb2 in the hematopoietic compartment leads to a phenotype that resembles the features of early human myeloproliferative disease.

T-1166

THE ROLE OF PRDM16 ISOFORMS IN HEMATOPOIETIC STEM CELL FUNCTION

Larry L. Luchsinger[1], Kenjiro Kamezaki[1], David Corrigan[1], Eric Schon[2], Hans Willem Snoeck[1]
[A]Columbia Center for Translational Immunology, [B]Neurology, [1]Columbia University, New York, NY, United States

Hematopoiesis is the continuous production of differentiated blood cells from hematopoietic stem cells (HSCs) located in bone marrow. HSCs can self renew and are multipotent. However, the mechanisms regulating these properties have not yet emerged. Treatments of disorders such as leukemia, lymphoma and myeloma have been successfully achieved through HSC transplantation. Hence advancing our understanding of HSC biology may improve targeted therapies to treat diseases of HSC origin. Findings by our lab and others have shown the transcription factor PRDI/RIZ1 domain molecule 16 (Prdm16) is specifically required for HSC maintenance and self renewal yet its molecular targets are unknown. Expression of mitochondrial genes, such as the brown adipose-specific thermogenic regulator uncoupling protein 1 (Ucp1), requires *Prdm16*. ***Therefore, we hypothesized that Prdm16 deletion alters mitochondrial function in HSCs.*** To evaluate mitochondria, lineage$^-$/cKit$^+$/Sca$^+$ (LSK) hematopoietic stem progenitor cells (HSPCs) and mouse embryonic fibroblasts (MEFs) stained with Mitotracker Red (MTR) revealed a filamentous network while *Prdm16*$^{-/-}$ cells displayed fragmented and dispersed globular mitochondria which showed defective fusion as observed in mitochondrial fusion assays. Evaluation of mitochondrial morphology in wild type (Wt) hematopoietic cell compartments revealed that HSC mitochondria, and to a lesser extent multiple myeloid progenitor (MPP), were hyperfused compared to differentiated lineages, suggesting a critical role for mitochondrial fusion in primitive hematopoietic cells. Together, these data show HSCs have hyperfused mitochondria and Prdm16 regulation of mitochondrial dynamics is critical for HSC maintenance. Regulation of mitochondrial dynamics relies on a balance in activity of the fission protein dynamin-related protein (Drp1) and the mitofusin (Mfn) family of outer mitochondrial membrane fusion proteins. Therefore, we tested the hypothesis that *Prdm16* regulates *Mfn* expression. *Mfn2*, but not *Mfn1* mRNA levels were decreased >80% in *Prdm16*$^{-/-}$ than in Wt MEFs. Similar data were obtained in using adult *Prdm16*$^{+/-}$ LSK cells. As expected, retroviral Prdm16 restored expression of *Mfn2* to Wt levels in MEFs. Chromatin immunoprecipitation (ChIP) of epitope-tagged Prdm16 constructs showed association of Prdm16 to the *Mfn2* locus in *Prdm16*$^{-/-}$ MEFs. These data suggest *Prdm16* regulates *Mfn2* expression and mitochondrial dynamics. Impaired fusion has been shown to result in abnormal mitochondrial metabolism. Therefore, we measured oxygen

consumption rate (OCR) and extracellular acidification rate (ECAR) which are proportional to the degree of OXPHOS and glycolysis. We found a strikingly lower baseline OCR in *Prdm16*$^{-/-}$. Conversely, ECAR was several folds higher in *Prdm16*$^{-/-}$ than in Wt MEFs, consistent with a >2-fold increase in lactate dehydrogenase (LDH) activity in *Prdm16*$^{-/-}$ MEFs relative to Wt control. These data are consistent with reduced mitochondrial respiration and a compensatory increase in glycolysis, a phenotype also observed in cells where *Mfn2* was knocked down. These data suggest *Prdm16*$^{-/-}$ MEFs have inefficient respiration. In conclusion, we identified *Mfn2* to be a novel target of Prdm16 regulation, the disruption of which alters mitochondrial dynamics. These findings may explain the loss of HSC maintenance and self renewal observed in *Prdm16*$^{-/-}$ HSCs and propose a critical role for mitochondrial dynamics in stem cell function.

T-1167

HEMATOPOIETIC STEM CELL HETEROGENEITY IS MAINTAINED BY DISTINCT ARTERIOLAR PERIVASCULAR AND ENDOSTEAL ZONES

Ryohichi Sugimura, Xi C. He, Aparna Venkatraman, John M. Perry, Linheng Li
Stowers Institute, Kansas City, MO, United States

Heterogeneity in either hematopoietic stem cells (HSCs) or their associated niches has been recognized in recent years; however, the relationship between the two and related properties remains largely unexplored. We attempted to characterize the function, location and metabolism of deeply quiescent HSCs and less quiescent HSCs. To determine cell-cycle frequency of such populations, we used CD49b, which is able to distinguish long-term (LT)-HSCs from intermediate-term (IT)-HSCs, to separate CD34^{-}Flk2^{-}LSK HSCs. We found that CD49b^{lo} HSCs enter into cell-cycle 1/100-120 days, while CD49b^{hi} HSCs do so 1/25-30 days according to H2B-GFP label regression assay. In the competitive reconstitution assay, CD49b^{lo} HSCs supported multipotent hematopoiesis for life-long (80% donor engraftment), while CD49b^{hi} HSCs supported hematopoiesis with 20% donor-engraftment and lost red blood cells and myeloid lineage by 20 weeks. CD49b^{lo} HSCs survived 5-fluorouracil (5FU) stress and underwent subsequent expansion to replenish CD49b^{hi} HSCs that were sensitive to 5FU and lost post treatment. Therefore, we demonstrated that CD49b^{lo} HSCs were deeply quiescent and functioned as a reserve population, while CD49b^{hi} HSCs were less quiescent and primed for action (primed HSCs). We then found that CD49b^{lo} reserve HSCs were enriched in the endosteal zone of the trabecular bone region, while CD49b^{hi} primed HSCs were enriched in the arteriolar (Tie2^{+}) perivascular zone. Furthermore, reserve and primed HSCs preferentially underwent anaerobic (p-PDH^{+} indicating glycolysis) and aerobic (Cox4-1^{+} indicating mitochondria-dependent) metabolism, respectively. This differential metabolic pathway was consistent with their hypoxic (GRP78^{+}) or hyperoxic microenvironment. We previously reported that deeply quiescent HSCs were maintained by noncanonical Wnt signaling through Frizzled (Fz) 8 in the endosteal zone (Sugimura *et al*., 2012. *Cell*). Now we show that another noncanonical Wnt receptor Fz5 maintains primed HSCs in the Fz5^{+}Nestin-GFP^{+}alpha-smooth muscle actin (αSMA)$^{+}$ cells in the arteriolar perivascular zone. Mechanistically, Fz5 regulates planar cell polarity (PCP) pathway and determines the polarity of the Cdc42-CXCR4 complex in primed HSCs. Thus CXCR4 engages with membrane-bound SDF1 in adjacent CXCL12-abundant reticular (CAR) cells. Loss of Fz5 results in random distribution of CXCR4 in primed HSCs and leads to HSC migration to the sinusoidal zone_leading to increased cell cycling coupled with loss of functional capacity. Thus, we establish the distinction between primed and reserve HSCs based on niche location, metabolism, signaling pathway regulation, and functional capacity.

T-1168

ELUCIDATION OF NOVEL GENETIC REGULATORS OF HEMATOPOIETIC STEM CELL DEVELOPMENT USING A ZEBRAFISH FORWARD GENETIC SCREEN

Julie R. Perlin[1], Logan Carr[1], Carolyn Wise[1], Weijun Pan[2], Leonard Zon[1]
[1]Hematology Oncology, Children's Hospital, Boston, Boston, MA, United States, [2]Institute of Health Sciences, Shanghai Institute of Life Sciences, Shanghai, China

Marrow transplantation of hematopoietic stem cells (HSCs) is an important therapy for patients with a variety of anemias, immunodeficiencies, and leukemias. The transplant relies on the homing and engraftment of donor HSCs to the recipient marrow, yet the genetics of such cell mobility are not understood. In development, HSCs arise from the aorta, enter circulation, and then undergo dynamic interactions with associated endothelial cells to form the blood stem cell niche. We have undertaken the first large-scale genetic screen to uncover pathways essential for HSC engraftment into the stem cell niche in the zebrafish. Over 50 mutants were found that were morphologically normal, but that had specific alterations in the expression of cmyb, a critical hematopoietic transcription factor, in the fetal liver equivalent of the zebrafish, or caudal hematopoietic tissue (CHT). cmyb is a conserved transcription factor expressed in hematopoietic progenitors, which is required for hematopoietic progenitor specification, maintenance, and differentiation. 5 mutant lines have been identified with a reduction of hematopoietic stem cells (HSCs) by reduced cmyb in the CHT. In order to determine whether this reduction is due to a lack of HSC production or a failure of HSCs to migrate towards and engraft into the niche, early hematopoietic progenitors were assayed by runx1 in situ hybridization. Mutants that produce HSCs that fail to engraft into the hematopoietic niche were prioritized for further study. We have developed a transgenic line that specifically labels HSCs and that allows for the first visualization of HSC-endothelial cell dynamics required for niche formation. Transgenic lines specifically labeling HSCs and endothelial cells have enabled us to observe engraftment defects in mutant embryos. These studies will provide insight into HSC development and hematopoietic niche formation, and the genes and pathways uncovered may be able to be manipulated to augment therapeutic marrow transplantation.

T-1171

NOVEL NANO-SCALE PHOSPHOPROTEOMIC IDENTIFICATION OF HEMATOPOIETIC PROGENITOR CELL MOBILIZATION PATHWAYS AND LEUKEMIA STEM CELL TARGETS

Leo Wang[1], Scott Ficarro[2], Jessica Sullivan[3], Amy Wagers[4]
[1]Dana-Farber/Children's Hospital Cancer Center, Harvard Stem Cell Institute, Boston, MA, United States, [2]Blais Proteomics Center, Dana-Farber Cancer Institute, Boston, MA, United States, [A]Joslin Diabetes Center, Harvard Stem Cell Institute, [B]Howard Hughes Medical Institute and Joslin Diabetes Center, Harvard Stem Cell Institute, [3]Harvard University, Boston, MA, United States

AML treatment often fails, in part because of the persistence of a small population of leukemia stem cells (LSCs) that are able to propagate disease but are resistant to standard chemotherapy. In order to target LSCs specifically, it is essential to identify pathways that are activated in LSCs but not in their nonmalignant counterparts, hematopoietic stem and progenitor cells (HSPCs). Pharmacologic mobilization of HSPCs mimics some of the characteristics of LSCs, including proliferation and migration. Importantly, however, mobilized HSPCs (mobHSPCs) retain their dependence on growth and stimulatory factors for maintenance; withdrawal of the mobilizing agent causes a swift contraction of the mobHSPC pool. Thus comparison of the resting bone marrow HSPC (bmHSPC) pool with the mobHSPC pool and with LSCs promises to identify unique pathways responsible both for blood cell mobilization and for leukemic transformation. This type of comparison has been performed at a transcriptional level and has yielded important findings, but cellular functions are ultimately effected by proteins. Additionally, mRNA abundance correlates poorly with levels of corresponding proteins and is insensitive to changes in functional activity of those proteins. Analysis of proteins themselves, therefore, is expected to yield critical insights that are not achievable through transcriptional analyses alone.
Only recently have methods been described that allow for detection of robust phosphoproteomes from small numbers of cells, enabling analysis of rare cell populations like HSPCs, which constitute only 0.01-0.1% of all bone marrow hematopoietic cells. Using flow cytometry, stable-isotope labeling, and a novel multidimensional nanoscale phosphoproteomic platform, we have successfully compared the phosphoproteomes of rigorously defined

bmHSPCs, mobHSPCs, and AML LSCs isolated by flow cytometry. Analysis of as few as $2x10^5$ flow-sorted cells by 3D RP-SAX-RP-MS/MS coupled to an Orbitrap Velos mass spectrometer resulted in detection of more than 3,600 unique phosphopeptide sequences. Hierarchical clustering and pathway analysis generated priority lists of candidate proteins more phosphorylated in the resting state (QuiCan), of mobilized state (MobCan) and malignant state (MalCan). Some of these, such as ribosomal protein S6 and PKCθ**,** have previously been described in the literature as important for hematopoietic progenitor function and mobilization. However, many novel candidates were also identified; the first of these has been validated using flow cytometry and protein biochemistry, and its role is being investigated *in vivo*.
We have developed a nanoscale phosphoproteomics platform able to analyze at high resolution small numbers of rare but biologically important cells. This technology has identified many proteins that are activated in HSPC mobilization, and many that are activated in leukemic transformation. Some of these candidates have already been validated, and more are undergoing validation. Further experiments will identify which of these pathways will be most attractive as a target for clinical therapies.

T-1172

ADENOSINE SIGNALING PROMOTES HEMATOPOIETIC STEM CELL PRODUCTION AND MIGRATION DURING ZEBRAFISH HEMATOPOIESIS

Lili Jing, Owen Tamplin, Ellen Durand, Margot Brandt, Leonard I. Zon
Hema/Oncology, Children's Hospital Boston, Boston, MA, United States

During embryogenesis, hematopoietic stem cells (HSCs) first emerge from hemogenic endothelium in the aorta-gonad-mesonephros (AGM) region, and then colonize the fetal liver and ultimately the bone marrow. The signaling pathways that regulate HSC development at different hematopoietic sites remain incompletely characterized. In zebrafish, the caudal hematopoietic tissue (CHT) is the functional equivalent of the mammalian fetal liver. The AGM-derived HSCs first seed at the CHT, expand and then migrate to the thymus and the pronephric kidney. To identify new modulators that directly enhance HSCs in the CHT, we performed a chemical genetic screen in which the embryos were exposed to individual compound from 48 hours post fertilization (hpf) to 72 hpf, and we identified that the adenosine pathway (including exogenous agonists and compounds that inhibit adenosine degradation) increased runx1/cmyb expression at the CHT. Using a hematopoietic stem/progenitor cell (HSPC) reporter line, we confirmed that the adenosine pathway increases the number of HSPCs that colonize the CHT. To examine if adenosine also expands HSCs at the AGM, we treated embryos with adenosine chemicals at an earlier time period (3-somite to 36 hpf) and found that adenosine also increased runx1/cmyb expression at the AGM. The effect was confirmed with adenosine receptor antagonists that decreased runx1/cmyb expression. Among the different adenosine receptors, the adenosine receptor A2B is expressed in endothelial cells in dorsal aorta and caudal vasculature. Knockdown of A2B strongly reduces HSPCs at AGM and CHT without affecting cardiovascular development and circulation. A panel of definitive linage specific markers are reduced in A2B knockdown embryos, supporting that adenosine pathway regulates functional HSPCs. Loss of A2B blocked the effect of adenosine on induction of HSPCs. Together, these data suggest that the adenosine pathway, acting through the A2B receptor, promotes HSC formation during development.

T-1173

TRACKING INDIVIDUAL HEMATOPOIETIC STEM CELL CLONES IN HUMANS BY RETROVIRAL TAGGING

Luca Biasco[1], Serena Scala[1], Cristina Baricordi[1], Francesca Dionisio[1], Andrea Calabria[1], Clelia Di Serio[2], Jason Gardner[3], Christof Von Kalle[4], Manfred Schmidt[4], Chiara Bonini[5], Luigi Naldini[1], Eugenio Montini[1], Alessandro Aiuti[1]
[1]San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Milan, Italy, [2]CUSSB, Università Vita-Salute San Raffaele, Milan, Italy, [3]GSK R&D, King of Prussia, PA, United States, [4]National Center for Tumor Diseases (NCT-DKFZ), Heidelberg, Germany, [5]San Raffaele Scientific Institute, HSR, Milan, Italy

Understanding how hematopoietic stem cells (HSC) generate and maintain an adequate pool of blood cells throughout human life is extremely relevant for the treatment of hematological disorders. However, information on HSC currently derives from in vitro assays or animal models but not from the direct tracking in humans of individual HSC clones. Upon retroviral gene transfer, transduced cells are univocally marked by vector integration sites (IS). Gene therapy (GT) clinical trials for ADA (adenosine deaminase) deficient-SCID and Wiskott-Aldrich Syndrome (WAS) based on the infusion of genetically engineered HSC, generate an hematopoietic system where vector-marked progenitors and blood cell progeny are traceable in vivo by IS analysis. This unique setting allows studying HSC dynamics at clonal level for the first time directly in humans. To this purpose we collected 32.363 IS from 3 WAS patients treated with lentiviral vector GT up to 1.5 years and 4.845 IS from 4 ADA-SCID patients treated with gammaretroviral (RV) GT up to 6 years after treatment. In WAS GT patients, by the measurement of clonal abundance through sequencing reads, we found an increase in the Shannon Diversity Index of clonal repertoires during the first year after GT. Self-limiting waves of hematopoietic output from transduced HSC were detected early after transplant, stabilizing at 12-18 months after GT. No aberrant clonal expansion was observed. Multipotent HSC could be tracked overtime by detecting identical IS in HSC/colony forming units and mature cells. Of note, we found the highest proportion of shared IS (38.5%) in BM clonogenic progenitors, an important validation of IS analysis consistency and of the colony assay as representative of in vivo HSC activity. Importantly, by mathematical estimators, we calculated a theoretical minimal number of about 7,500 transduced active stem cells corresponding to about 1 in 3x10e5 infused CD34+ cells. In ADA-SCID patients treated with RV-GT we showed that identical IS are consistently detected at multiple lineages level several years after GT. Since RV transduction occurs only in actively replicating cells, this finding provides the first evidence that in vitro stimulated HSC can retain long-term activity in humans. By semi-quantitative PCRs on specific vector-genome junctions we tracked the dynamics and activity of specific HSC clones in these patients, showing a fluctuating but consistent output of marked HSC over a period of 5 years. Notably, we were also able to apply network-based statistical approaches to test the validity of the different proposed models of hematopoietic hierarchy through IS similarity among lineages.
We also analyzed IS in T cell subtypes from ADA-SCID patients who received infusions of gene-corrected mature lymphocytes. Strikingly, we found that transduced T cells with an apparent naïve phenotype retained their plasticity, as they share the highest number of IS with other T subpopulations, while still surviving in vivo 10 years after infusion. These studies allowed us to track the fate of recently defined T memory stem cells (TSCM) carrying naïve plasticity and memory survival. In summary, our IS studies allow defining crucial stem cell properties in vivo in humans providing new information with profound impact on the design of therapeutic strategies for hematological diseases and tumors.

T-1174

RETINOIC ACID SIGNALING INHIBITION DURING HUMAN PLURIPOTENT STEM CELL DIFFERENTIATION PROMOTES HEMOGENIC MESODERM SPECIFICATION AND HEMATOPOIETIC STEM/PROGENITOR CELL MAINTENANCE

Roger Rönn[1], Carolina Guibentif[1], Roksana Moraghebi[1], Patricia C. Guerrero[2], Bradley Garcia[3], Ewa Sitnicka[2], Niels-Bjarne Woods[1]

[A]Gene Therapy and Molecular Medicine, [B]Hematopoietic Stem Cell Laboratory, [1]Lund University, Lund, Sweden, [2]Primorigen Biosciences, Madison, WI, United States

Hematopoietic stem and progenitor cells generated from patient derived induced pluripotent stem (iPS) cells could provide an unlimited supply of HLA matched transplantable cells for the treatment of both hematological disorders and malignancies. Retinoic acid (RA) is a key morphogen involved in the establishment of various cell-fates in the growing embryo. The establishment of certain tissues, including mesodermal derivates such as the somites, heart, and blood depends on restricted exposure to RA during embryonic development. By using an inhibitor of cellular RA synthesis together with our iPS-to-blood differentiation system we report enhanced generation of human hematopoietic progenitors cells (CD45/43+CD34+), and cells with an adult hematopoietic stem cell phenotype (CD45/43+CD34+CD38-CD90+CD45RA-), with myeloid and lymphoid differentiation capacity. RA inhibition increased the output of cells possessing an adult HSC surface phenotype by 2.7-fold (p-value: 0,006, n=6) com-

pared to DMSO control, and increased the number of clonogenic progenitors (CFUs) by 2-fold (p-value: 0,022, n=6). This improvement is consistent when using both a human ES line (Hues3) and a human iPS-line (RB9-CB1, generated from cord blood). Conversely, and in support of our findings, directly adding RA was found to severely decrease the blood generation efficiency of our protocol, with the more primitive progenitor fraction (CD45/43+CD34+CD38-CD90+CD45RA-) significantly decreased even at lower concentrations of RA. When comparing the colony forming potential of sorted hematopoietic progenitors, RA inhibition enabled the generation of CFU-forming cells at efficiencies directly comparable to that of CB, with colony counts being significantly higher per sorted progenitor than the DMSO control, thus suggesting increased preservation of functional CFU-forming cells. RA inhibition increased the number of iPS-derived CD45/43+CD34+ cells capable of differentiation into the lymphoid lineage, generating phenotypic T-, B-, and NK-cells after sub-culture on OP9 stroma. The observed increase in more primitive hematopoietic progenitors (CD45/43+CD34+CD38-CD90+CD45RA-) without any significant increase in the total number of blood cells (CD45/43+) suggest that RA inhibition act to maintain the more primitive progenitor fraction. However, RT-qPCR analysis of key developmental genes during the differentiation process revealed improved commitment and differentiation of the pluripotent stem cells towards hematopoietic mesoderm at several distinct developmental stages including increased generation of mesoderm (BRACHYURY), decreased amounts of later cardiogenic mesoderm (NKX2.5), and enhanced expression levels of genes known to be relevant for definitive hematopoiesis and hemogenic endothelium (RUNX1, FLK1, APLNR, PDGFRa). Together, these findings indicate that inhibition of RA signaling act to both enhance the developmental commitment towards hematopoietic mesoderm, and to improve the maintenance of newly emerged blood cells. We propose that control over RA signaling can, in addition to the already established roles of other key factors and cytokines, allow for better developmental specification towards various cell lineages in the in vitro setting.

T-1175

INTEGRIN BETA3 BIDIRECTIONALLY REGULATES LONG-TERM REPOPULATING ACTIVITY IN MURINE HEMATOPOIETIC STEM CELLS

Terumasa Umemoto[1], Yoshiko Shiratsuchi[1], Jun Ishihara[1], Sung-Joon Park[2], Kenta Nakai[2], Masayuki Yamato[1], Teruo Okano[1]

[1]Institute of Advanced Biomedical Enginerring and Science, Tokyo Women's Medical University, Tokyo, Japan, [2]Human genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Hematopoietic stem cells (HSCs) reside in a specialized microenvironment or “niche” within the bone marrow (BM), where their self-renewal, differentiation, and quiescence are regulated. We previously demonstrated that integrin αvβ3 (CD51/CD61) is highly expressed in murine CD34-KSL HSCs (CD34- c-Kit+ Sca-1+ Lin-), but not in CD34+KSL HSCs (Umemoto et al., J. Immunol, 2006). Further, integrin β3 signaling contributes to the maintenance of the long-term repopulating (LTR) activity of HSCs in vivo and in vitro, which is dependent on the presence of thrombopoietin (TPO) (Umemoto et al., Blood, 2012). Although our previous finding has suggested that integrin β3 functions as a positive regulator of HSC activity in the BM niche, in this study, we demonstrate a novel role of integrin αvβ3 in the regulation of HSCs.

Recently, we detected that triiodothyronine (T3), a thyroid hormone, could suppress the LTR activity of HSCs by means of transplantation assays using HSCs cultured in the presence or absence of T3. Interestingly, this negative effect of T3 is enhanced by specific ligation of integrin β3 via the extracellular matrix protein, even in the presence of TPO. In addition, integrin β3 signaling had little influence on HSC number when cultured in the presence of T3. These results indicate that T3 reverses the previously reported positive effect of integrin β3 on the LTR activity of HSCs and that integrin β3 signaling preferentially contributes to enhance the negative effect of T3 rather than to maintain LTR activity by collaborating with TPO. To clarify the influence of T3 on HSCs, we compared the gene expression profiles of untreated and T3-treated HSC globally by using mRNA sequences, and then carried out statistical analyses focused on transcription factor binding motifs within the promoter regions of the genes. The expression level of some of the analyzed genes is changed by T3 treatment, suggesting that T3 contributes to the enhancement of STAT1-dependent gene expression in HSCs. Excess activation of STAT1 reportedly diminishes LTR

activity of HSCs. In addition, we also confirmed that IFNγ, an activator of STAT1, showed the negative influence on the maintenance of LTR activity in HSCs. Moreover, this suppressive effect of IFNγ on LTR activity was also enhanced by integrin β3 signaling, similar to that of T3. These data suggest that STAT1 is involved in the T3-mediated reversal of integrin function.
Collectively, our results indicate that integrin β3 signaling in HSCs contributes to the suppression of the LTR activity of HSCs in the presence of T3 or IFNγ, possibly through STAT1-dependent control of gene expression. Thus, given our previous report that integrin β3 signaling in HSCs exerts the positive effect on LTR activity by collaborating with TPO, integrin αvβ3 on HSCs seems to be involved in the bidirectional regulation of their LTR activity in response to surrounding environments. Further, integrin αvβ3 may play a key role in the regulation of HSC functions and/or maintaining the stemness within the BM niche.

T-1176

DEFINING THE MOLECULAR BASIS FOR REVERSIBLE SUPPRESSION OF HEMATOPOIETIC STEM CELLS IN LEUKEMIC MARROW

Hui Cheng, Yanfeng Liu, Yakun Pang, Sha Hao, Shihui Ma, Yingxu Shi, Jing Xu, Jinhong Wang, Guoguang Zheng, Weiping Yuan, Tao Cheng
Institute of Hematology, Tianjin, China

The response of hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs) to stress or disease conditions has been gaining increased attention in recent years. Our previous study demonstrated that HSCs/HPCs were reversibly suppressed in an irradiated acute T lymphoblastic leukemia (T-ALL) mouse model (Hu X et al, *Blood 2009*). This current work aims to further examine the kinetics of normal HSCs and HPCs in acute myeloid leukemia (AML) and to define a common molecular basis of suppressed HSCs in AML and ALL.
To this end, we first established an AML model using the human MLL-AF9 fusion gene in non-irradiated mouse recipients. Normal hematopoiesis was progressively suppressed during leukemia development in this model, with the leukemic environment imposing different effects on LKS^+ HSCs and LKS^- HPCs in the bone marrow (BM). The frequency of normal LKS^- HPCs in the leukemic BM increased modestly and then was exhausted during leukemogenesis whereas the frequency of normal LKS^+ or $LKS^+CD34^-Flk2^-$ cells progressively increased from day 7 to day 21. The absolute numbers of both HSCs and HPCs in the BM decreased during leukemia development. In contrast, the frequencies and absolute numbers of HSCs and HPCs in the leukemic spleen increased from day 7 to day 14, and then dropped to normal levels at day 21. Cell cycle status was further assessed. More HSCs and HPCs were accumulated in the G0 phase in leukemic BM during leukemia development and as a result, LKS^+ or LKS^+CD34^- HSCs in the late stage of leukemia were kept in a nearly non-proliferative status. Interestingly however, HPCs had an increased proliferative rate and HSCs showed a normal cell cycle profile in the leukemic spleen. In accordance with the increased frequency of primitive cells, the normal $CD45.1^+$ cell population from leukemic BM had a significantly higher long-term engraftment capacity than that from normal BM at day 14. However, no significant difference was observed in the competitive repopulation assay when the long-term repopulating HSCs were transplanted, indicating equal reconstitution potential of the HSCs (average function per HSC) from leukemia versus control mice. Furthermore, we also sorted the $CD45.1^+LKS^+$ population from control or leukemia mice at different time points for the gene expression profiling analysis. Three genes (Maff, Hey1, Egr3) were selected for a series of functional validations. Using the zebrafish knock-down assay, we found that all three genes seemed to be required for definitive hematopoiesis. Upon forced expression of these genes in mouse HSCs by retroviral transduction, Egr3 restricted the proliferation of HSCs likely via up-regulation of the CDK inhibitors, p18 and p19, whereas Maff and Hey1 were able to enhance the function of HSCs, thus suggesting that these genes play distinct roles in inhibiting or protecting HSCs during the expansion of leukemic cell populations.
In summary, our current study reveals a molecular basis for the reversible suppression of normal HSCs during the expansion of leukemic cells in the BM, thereby having implications for the development of new therapeutic strategies for leukemia.

T-1177

PTEN LOSS IN THE BONE MARROW LEADS TO GCSF MEDIATED HSC MOBILIZATION.

Melania Tesio[1], Gabriela Oser[1], Irene Baccelli[1], Hong Wu[2], Joachim R. Göthert[3], Scott C. Kogan[4], Andreas Trumpp[1]
[1]German Cancer Research Center, Heidelberg, Heidelberg, Germany, [2]Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, Los Angeles, CA, United States, [3]University Duisburg-Essen, Essen, Germany, [4]USCF Medical Center, San Francisco, CA, United States

The phosphatase and tumor suppressor PTEN inhibits the phosphoinositol-3-kinase (PI3K) signaling pathway and plays a key role in cell growth, proliferation, survival and migration. Pten conditional deletion using MxCre or Scl-CreERT2 leads to splenomegaly and leukemia formation, which occurs after the re-location of normal HSCs from the bone marrow to the spleen. Unexpectedly, dormant HSCs in the bone marrow do not enter the cell cycle upon PTEN loss nor do they lose self-renewal activity or are exhausted. Instead, Pten deficiency causes an up-regulation of the PI3K pathway in myeloid cells, but not in HSCs. Strikingly, myeloid cells secrete high levels of G-CSF upon Pten loss leading to the mobilization of HSCs from the bone marrow and accumulation in the spleen. Following deletion of Pten in mice lacking G-CSF the splenomegaly, myeloproliferative disease and splenic HSCs accumulation are rescued. Our data show that while PTEN has little if any role in HSCs, it is essential to prevent overt G-CSF production by myeloid and stromal cells which otherwise causes HSCs to re-locate to the spleen followed by lethal leukemia initiation.

T-1178

EXPANSION AND ORDERLY DIFFERENTIATION OF MEGAKARYOCYTIC PROGENITOR CELLS GENERATED FROM CORD BLOOD HEMATOPOIETIC STEM/PROGENITOR CELLS AND ITS PHASE I/II CLINICAL TRIALS

Xuetao Pei
Inst of Transfusion Medicine. Academy of Military Medical Sciences, Beijing, China

Thrombocytopenia is a common and potentially fatal complication of high-dose chemotherapy and hematopoietic stem cell transplantation. Infusion of platelets from unrelated donors is currently the only effective treatment to prevent fatal hemorrhage. Hematopoietic stem cells (HSCs) from bone marrow (BM), cord blood (CB), and peripheral blood (PB) can be used to generate functional hematopoietic progenitor cells, including megakaryocytic progenitors (MPs), megakaryocytes, and platelets. Umbilical cord blood is an abundant source of HSCs. Cord blood is also highly enriched in committed hematopoietic progenitor cells, including those of the megakaryocytic lineage. In vitro large scale production of hematopoietic progenitor cells from cord blood could represent an effective blood cell substitute. In the present study, our objective was to determine the safety and feasibility of ex vivo generated hematopoietic progenitor cells (HPCs) in patients with hematological malignancy. Based on promising results of our preclinical study, state food and drug administration (SFDA) of China approved our group to conduct a clinical trial of HPCs injection to patients with hematologic malignancy. We investigated the feasibility of large-scale expansion, orderly differentiation and infusion of cord blood-derived HPCs in the patients with advanced hematological malignancyes. No adverse effects were observed in patients who received ex vivo-generated cells. Further, a moderate effect on platelet recovery was also observed. Administration of cord blood-derived HPCs appeared safe and feasible for treatment of thrombocytopenia after chemotherapy.

T-1181

EFFECT OF MESENCHYMAL STEM CELL TO SUPPORT GAMMA IRRADIATED HUMAN UMBILICAL CORD BLOOD CD133+ CELLS

Roya Rafiee[1], Marzieh Ebrahimi[2], Nasser Aghdami[3], Hamid Gourabi[1]
[A]Genetics, [B]Cancer and Hematopoietic Stem Cells, [C]Regenerative Medicine, [1]Royan Institute, Tehran, Iran, Islamic Republic of

The recovery of organs and tissues after irradiation process are usually depend on resident stem cells re-population in target organ. Hematopoetic Stem Cells (HSCs) and Mesenchymal Stem Cells (MSCs) constitute two major stem cells populations in bone marrow with more primitive phenotype sub-population of bone marrow mononuclear cells and adherent fibroblast-like cells, respectively. Interaction of these two populations have significant role in maintaining and reconstruction of hematopoesis system.Umbilical cord-derived stromal cells were applied in order to recovery of irradiated CD133+ which derived from human umbilical cord-blood in a serum-free liquid culture supplemented with combination of stem cell factor, thrombopoietin and Flt-3. Post irradiation analysis were performed for human irradiated and non-irradiated CD133+ cells at the day of experiment and also seven days after irradiation in presence/absence of MSCs. Lymphoid and myeloid-erythroid lineage markers (CD33, CD19), expression of adhesion molecule (CXCR4), HSCs markers (CD133, CD34 and CD133/34), and DNA breaks were analyzed by flowcytometry technique. The colony-forming cells were assessed by methylcellulose culturing method using MethoCult. Expansion rate of cells were evaluated by cell counting.
Our results demonstrated that MSCs can support expansion of human irradiated CD133+ with ratio of 1.92 fold. Their colony-forming potential was 2.12 fold with an increasing ratio to 2.14 fold in Granolocyte-Macrophage colonies (GM) and 1.6 fold in MIX colonies (GM-BFU). Presence of MSCs was caused shifting to myeloid-erythroid lineage with expression of CD33 (19.7 ± 12%). Co-cultured of human irradiated CD133+ with MSCs were shown the expression of CD 133 (5.36 ± 1.57%), CD34 (2.5 ± 0.75%) and CD133/34 (1.5 ± 0.5%). Interestingly, DNA breaks repair was increased to 7.5 folds co-cultured with MSCs. Expression of CXCR4 did not reveal a significant expression in both groups.
Direct contact of human irradiated CD133+ with MSCs preserve not only the expansion, and function of human irradiated CD133+ cells, but also shifting towards to mylo-erythroid lineage. MSCs promotes recruitment of DNA repair system and deletion of free-radical which resulted from ionizing radiation.

T-1182

OSTEOBLAST ABLATION FROM THE BONE MARROW ENHANCES SHORT-TERM HEMATOPOIETIC STEM CELLS WITHOUT ALTERING LONG-TERM SELF-RENEWING HEMATOPOIETIC STEM CELL POPULATIONS

Marisa Bowers, Ho Yin-Wei, Ravi Bhatia
Beckman Research Institute, City of Hope, Duarte, CA, United States

The bone marrow (BM) microenvironment plays a critical role in supporting and regulating normal hematopoiesis. Hematopoietic stem cells (HSCs) are reported to reside in proximity to endosteal osteoblasts (OBs) in the BM, and OBs are hypothesized to provide a HSC niche. However, other studies suggest that perivascular mesenchymal cells or endothelial cells provide HSC niches, and the specific role of OBs in maintaining HSCs is not well understood. We use a conditional OB ablation mouse model (Col2.3Δtk) to examine the role of OBs in hematopoiesis and HSC function. In this model a truncated version of herpes simplex virus thymidine kinase (Δtk) is specifically expressed in OB. Daily intraperitoneal (IP) administration of ganciclovir (GCV) leads to interaction with Δtk, producing a toxic DNA base analogue that results in specific killing of OBs. To facilitate tracking of OB ablation, we crossed the Col2.3Δtk mice with Col2.3GFP mice that specifically express GFP in OBs. Through immunofluorescent, immnunohistochemical and flow cytometry analysis, we confirmed GCV administration ablated endosteal OBs in our model system. OB ablation resulted in a decrease in overall BM cellularity (Δtk+ 3.7e7±3.0e6, Δtk- 4.8e7±3.8e6, p=0.04), but did not alter cellularity of the spleen (SP) (Δtk+ 5.1e7±5.3e6, Δtk- 6.3e7±7.4e6, p=0.19). Flow cytometric analysis of hematopoietic populations showed that OB ablation significantly increased phenotypic long-term HSC (LT-HSC) populations (Lin-Kit+Sca-1+Flt3-CD150+CD48-) in both the BM (cell number: Δtk+ 6490±1315, Δtk- 4236±922; percentage: Δtk+ 0.017%±0.003, Δtk- 0.009%±0.002; p=0.03) and SP (cell number: Δtk+ 980±473, Δtk- 96±40; percentage: Δtk+ 0.002%±0.0001, Δtk- 0.0002%±0.001; p=0.04). OB ablation also leads to significant increases in common myeloid progenitor (CMP) (cell number: Δtk+ 145114±43608, Δtk- 82200±26754; percentage: Δtk+ 0.26%±0.056, Δtk- 0.11%±0.021; p=0.002) and granulocyte/monocyte progenitor (GMP) (cell number: Δtk+ 51411±17349, Δtk- 20206±9279, p=0.003; percentage: Δtk+ 0.09%±0.03, Δtk- 0.31%±0.01, p=0.02) populations in the SP, but does not alter other hematopoietic populations in BM, SP or PB. To determine the stem cell potential of phenotypically defined LT-HSCs in our model we performed limiting-dilution competitive repopulation assays. LT-HSCs from OB ab-

lated mice demonstrated a higher frequency of short-term repopulating cells (5 and 10 weeks) compared to LT-HSC from non-ablated mice (5 weeks Δtk+:Δtk-=3.51, 10 weeks Δtk+:Δtk-=4.99) but similar long-term engraftment (15 weeks; Δtk+:Δtk-=1.01). Transplantation of BM cells from primary transplant recipients into secondary recipients demonstrated that LT-HSCs from OB ablated mice have similar long-term engraftment potential to those from non-ablated mice after second transplant. These results suggest that the increased numbers of phenotypic LT-HSCs in OB ablated mice may represent the expansion of a HSC pool with short-term repopulating capacity, but that HSCs with long-term repopulating and self-renewing capacity remain unchanged in OB ablated mice. Collectively, these studies indicate that OB are not an essential niche component for long-term repopulating, self-renewing HSCs, but may restrict generation and/or expansion of short-term HSCs within the BM.

T-1183

ESTABLISHMENT OF SERUM-FREE CULTURE SYSTEM OF PERIPHERAL BLOOD MONONUCLEAR CELLS TO POTENTIATE VASCULAR REGENERATION

Haruchika Masuda[1], Rica Tanaka[3], Satoshi Fujimura[3], Masakazu Ishikawa[4], Hiroshi Akimaru[5], Tomoko Shizuno[1], Atsuhiko Kawamoto[2], Takayuki Asahara[1]

[A]Regenerative Medicine, [B]Vascular Regeneration Research Group, [1]Tokai Univ. School of Med., Isehara, Japan, [2]Plastic and Reconstructive Surgery, Juntendo Univ. School of Med., Tokyo, Japan, [3]Medicine, Case Western Reserve University, Cleveland, IL, United States, [4]Vascular Regeneration Research Group, Institute of Biomedical Research and Innovation, Kobe, Japan

BACKGROUND: Apheresed cell therapy for vascular regeneration targeting ischemic diseases using peripheral blood (PB) mononuclear cells (MNCs) or PBCD34+ cells underwent G-CSF administration, has been clinically developed. Here, we tried to investigate the therapeutic potential of MNCs isolated from intravenously aspirated PB, operated the recently developed serum-free culture (SFC) of stem cell fraction for augmentation of vascular regeneration.
METHODS and RESULTS: SFC were performed for PBMNCs isolated from the healthy human subjects (N= 18), using StemLine II containing SCF, TPO, Flt-3 ligand, VEGF and IL-6 for 7 days. EPC- colony forming assay unveiled the outstanding expansion of vasculogenic definitve colony forming cells in SFC cells at 42 fold in the equivalent cell No. of PBMNCs and 19 fold per original 100 ml PB. Moreover, flow cytometry of SFC cells disclosed the anti-inflammatory macrophage polarization of M1 (CCR2+ cell) to M2 (CD206+ cell) at 2.1 x10-3 fold of PBMNCs in M1/M2 ratio.
In real time qPCR, the gene expression of growth factors/cytokines in SFC cells was predominantly upregulated (fold increase = 4.2 or 2.4 for VEGF-B or angiopoietin-1; 35.9, 6.3 or 5.4 for Leptin, IL-8 or IL-10), inversely that of IL-1β or TGF- β downregulated (fold decrease = 0.23 or 0.44 for IL-1β or TGF- β).
Laser doppler analysis at day 20 post the intramuscular cell transplanattion (Tx) (1 x104 cells /mouse= equivalent to the cell No. acquired from less than 100 ml human PB) at the next day of ischemic surgery presented that SFC cell-Tx significantly recovered the blood flow ratio of ischemic/healthy hindlimb at 1.8 fold vs PBMNC-Tx, where the ratio was equivalent to non cell-Tx (N= 12). The assessment of ischemic hindlimb disclosed that SFC cell-Tx increased isolectin B4-FITC+ capillary density at 3.4 fold vs PBMNC-Tx. Further, the recruitment of α SM actin+ pericytes in SFC cell-Tx was also predominantly detected at 2.0 fold vs PBMNC-Tx.
CONCLUSION: SFC potentiates the vascular regeneration ability of naïve PBMNCs isolated under venous aspiration via the orchestration of promoted effects for arteriogenesis, anti-inflammation, as well as angiogenesis, thereby providing a practical therapeutic application for ischemic diseases.

T-1184

WNT/β-CATENIN AND MTOR SIGNALING REGULATE MAINTENANCE OF HUMAN AND MOUSE HEMATOPOIETIC STEM CELLS

Michelle Nguyen-McCarty, Jian Huang, Peter Klein
University of Pennsylvania, Philadelphia, PA, United States

Hematopoietic stem cells (HSCs) possess the dual capacities to self-renew and to differentiate into all blood cells. The molecular signals regulating HSC homeostasis are incompletely understood. We thus aimed to define regulators of HSC maintenance. We have found that knockdown of *glycogen synthase kinase-3* (*Gsk-3*) in bone marrow (BM) results in transient expansion of phenotypic and functional HSCs, followed by depletion of HSCs in long-term serial transplants. We find that inhibition of GSK-3 activates both Wnt/β-catenin and mechanistic target of rapamycin (mTOR) signaling in hematopoietic cells. Conditional knockout of *β-catenin* blocks initial HSC expansion in *Gsk-3*-depleted BM, while inhibition of mTOR prevents subsequent HSC depletion. Our working hypothesis is that GSK-3 regulates both self-renewal and differentiation of HSCs by regulating two antagonistic pathways. GSK-3 inhibition of canonical Wnt/β-catenin signaling inhibits HSC self-renewal, while GSK-3 inhibition of mTOR inhibits lineage commitment. Inhibition of GSK-3 activates both β-catenin and mTOR, promoting both self-renewal and lineage commitment pathways. The combination of GSK-3 and mTOR inhibitors may therefore simultaneously suppress the commitment pathway and promote self-renewal in HSCs. In support of this model, we have recently shown that HSCs cultured in serum-free, cytokine-free medium supplemented with both GSK-3 and mTOR inhibitors reconstitute the hematopoietic systems of lethally irradiated mice. This is the first time HSCs have been maintained *ex vivo* in the absence of hematopoietic cytokines. We are now identifying downstream mediators of Wnt/β-catenin and mTOR signaling to define the mechanism by which these pathways regulate HSC homeostasis.

T-1185

HUMAN REPORTER MESENCHYMAL STROMAL CELLS (MSC) FOR HIGH-THROUGHPUT-SCREENING OF INTERACTIONS BETWEEN BIOACTIVE MATRICES AND MSC

Regina Duryagina[1], Konstantinos Anastassiadis[3], Stefan Gramm[4], Manfred Maitz[4], Susan Schneider[5], Manja Wobus[1], Sebastian Thieme[2], Sebastian Brenner[2], Carsten Werner[4], Martin Bornhäuser[1]

[A]Medical Clinic and Policlinic, [B]Department of Pediatrics, [1]University Hospital Dresden, Dresden, Germany, [2]Center for Regenerative Therapies Dresden, Dresden, Germany, [3]Leibniz Institute of Polymer Research, Max Bergmann Center of Biomaterials Dresden, Dresden, Germany, [4]BioInnovations Center Technical University Dresden, Dresden, Germany

Recent clinical studies have shown that ex-vivo expansion of hematopoietic stem and progenitor cells (HSPC) on mesenchymal stromal cells (MSC) leads to a superior amplification of $CD34^+$ HSPC and can be successfully transplanted. However, to date approaches describing MSC-based expansion of HSPC recruit unmanipulated naïve MSC, while further induction of their HSC-supportive potential can additionally improve outcomes of HSPC expansion. ECM proteins and signalling molecules besides affecting HSPC are capable of modulating gene expression profile of MSC. We suppose that ECM components in combination with signalling proteins can influence HSPC-supportive potential of MSC. Therefore, we aimed at identifying ECM and signalling proteins in a combinatorial fashion in order to screen their potential capability of inducing the expression of HSPC-supportive genes in MSC. For this purpose we initially generated dual-reporter constructs encoding two secreted reporter proteins: Gaussia luciferase under the control of human Angiopoietin-1 (Ang-1) or human stromal cell-derived factor-1 (SDF-1) promoters and secreted alkaline phosphatise (SEAP) under the control of a constitutive promoter to normalize promoter activity. Next, we stably transfected immortalized human bone marrow-derived MSC with Ang-1 and SDF-1 dual-reporter constructs to generate Ang-1 and SDF-1 reporter MSC, respectively. Established high-sensitive secreted dual-reporter systems enabled subsequent continuous monitoring of Ang-1 and SDF-1 gene expression in MSC cultured for 8 days on ECM/signalling protein microarray plates. For the generation of these plates we used an automated workstation to immobilize varying amounts of ECM proteins, namely collagen I, fibronectin, laminin, and vitronectin, alone and in combination with signalling proteins, in particular Jagged-1, N-cadherin, or Wnt3a. Overall more that 100 conditions were available in triplicates on a 384-well plate for each type of reporter MSC. These reporter arrays showed that Ang-1 and SDF-1 expression can be induced by different ECM and morphogen combinations. Thus, fibronectin alone and its combination with all signalling proteins induced the highest level of Ang-1 expression compared to all other conditions, while SDF-1 expression was maximally induced by laminin and its combination with the aforementioned signalling proteins. The most relevant findings of the array experiments could also be

confirmed by quantitative PCR. Furthermore, continuous monitoring of gene expression revealed combinations, in particular with collagen, capable of only short-term induction of gene expression. Taken together, the newly designed dual-reporter array based on transduced MSC allows for high-throughput-screening of ECM and signalling proteins inducing expression of HSC-supporting genes in MSC in a time and dose dependent manner.

T-1186

MESENCHYMAL STEM CELLS AS GRAFT VERSUS HOST DISEASE PROPHYLAXIS IN TRANSPLANT OF THALASSEMIA MAJOR PATIENTS: AN EXPERIENCE FROM INDIA

Ashis Mukhopadhyay, Jayasri Basak, Soma Mukhopadhyay, Argha Nandy, Ujjal kanti Roy
Netaji Subhas Chandra Bose Cancer Research Institute, Kolkata, India

Background: Mesenchymal Stem Cells (MSCs) have unique characteristics such immune suppression by inhibiting T cell proliferation and acceleration of hematopoietic stem cell engraftment. Soluble factors and cells are implicated in the MSC-mediated T cell suppression and numerous clinical trials using MSCs to prevent graft-versus-host disease (GVHD) have been reported. MSCs are suggested to suppress acute GVHD without impairing graft-versus-leukemia effects. Our aim is to see the effect of MSCs in GVHD prophylaxis and result of thalassemia major patients.
Material & Method: We selected 10 thalassemia major patients whose umbilical cord blood transplants were performed at bone marrow transplant unit of Netaji Subhas Chandra Bose Cancer Research Institute, Kolkata, India. The age range was 4-15 years (mean 8.5). Sibling cord blood was used as stem cell source. In 5 patients we used routine GVHD prophylaxis (methotraxate, cyclosporine and methlprednisolone). In another 5 patients we added additional cord blood MSCs. MSCs from sibling cord blood were cultured and administered to the recipients at doses of 0.8-1.3×106/kg when the blood count indicated recovery.
Result: GVHD of stage II-IV developed in 4 (80%) out of 5 thalassemia transplants where routine GVHD prophylaxis was used. On the contrary only 1 patient (20%) developed grade III, 1 patient grade II and 3 patients developed grade I GVHD where additional MSC were used as GVHD prophylaxis. There were no difference in the graft rejection rate, chronic GVHD development, or infectious complication. There was no mortality in any of the patients in both the groups. There was a rejection (20%) of the graft in the group where no MSC was used.
Conclusion: We can conclude that MSC can be safe and effective method of GVHD prophylaxis in thalassemia transplants. The number is very small. We intend to continue the programme more in thalassemia transplants as well as in other allogenic transplants.

T-1187

IN-VIVO SINGLE CELL RNA-SEQ ANALYSIS OF OSTEOLINEAGE CELLS WITHIN THE HSPC NICHE IDENTIFIES INTERLEUKIN-18 AS A NOVEL HEMATOPOIEITIC REGULATOR

Lev Silberstein[1], Peter Kharchenko[2], Masatake Osawa[1], Charles Lin[1], Francois Mercier[1], Youmna Kfoury[1], Christina Lo Celso[3], David T. Scadden[1]
[1]Massachusetts General Hospital, Boston, MA, United States, [2]Harvard Center for Biomedical Informatics, Boston, MA, United States, [3]Imperial College, London, United Kingdom

Despite substantial experimental evidence suggesting that osteolineage cells (OLCs) regulate HSPC behavior, the molecular nature of OLC-HSPC cross-talk remains largely unknown. We hypothesized that OLCs adjacent to transplanted HSPC represent a distinct “niche OLC” population, which is enriched for HSPC-regulating molecules. We set out to define the molecular signature of this population in-vivo by comparative transcriptome analysis of the individual col2.3GFP+ OLCs located proximally (<2 cell diameters) and distally from a single transplanted HSPC.
In order to address this question, we established a novel method of targeted single cell retrieval, which enabled micromanipulator-assisted aspiration of HSPC-proximal and HSPC-distal OLCs into a glass micropipette directly from live unfixed sections of neonatal mouse bone, followed by single cell RNA-Seq analysis.

Our data revealed that HSPC-proximal OLCs had a distinct transcriptional signature, both at the level of top 200 differentially expressed genes and genome-wide, as determined by a blind classifier software. Gene set enrichment analysis showed a significant increase for genes associated with cell migration (p=0.003) and angiogenesis (p=0.008); moreover, a literature-derived “HSPC-niche” gene set (CXCL12, angiopoietin 1, placental growth factor, VCAM-1, IL-7, Wnt-4) was highly upregulated in the HSPC-proximal OLCs (combined p=2.1-10). These data suggest that HSPC-proximal OLCs represent a functionally distinct subset of OLCs with HSPC regulatory function.
We further investigated the role of two individual genes which were preferentially expressed in the HSPC-proximal cells: cell adhesion molecule Embigin (Emb) and proinflammatory cytokine IL18. Using antibodies against Embigin and VCAM-1, we isolated a small (3.4%) subset of Embhigh col2.3GFP+ OLCs from adult mouse bones and found that the Q-PCR profile of these cells recapitulated that of an individually picked single HSPC-proximal OLCs, in particular with regard to quiescence-inducing molecules CXCL12 and angiopoietin-1. In col2.3PPR mice, we found that a 2 -fold reduction in Embhigh col2.3GFP+ OLCs was associated with an increase in proportion of LT-HSCs in G2/S/M phase of cell cycle, indicating that HSCP-proximal cells are involved in control of HSPC quiescence.
Consistent with this observation, we identified IL18 as a novel regulator of HSPC quiescence. Under homeostatic conditions, IL18 KO mice had a decrease in the proportion of HSPCs in the G0 phase of the cell cycle (82% vs 87%), which was associated with a small (1.4-fold) reduction in the frequency of HSPCs in the bone marrow, presumably due to their loss through excessive proliferation. Upon transplantation, IL18-deficient microenvironment conferred significantly faster short-term reconstitution ability to WT bone marrow cells.
We conclude that our approach identified a rare population of OLCs with HSPC niche function and uncovered a previously unknown role of IL18 as a hematopoietic regulator; our data suggest that attenuating IL18 signaling may be clinically relevant in accelerating post-transplantation bone marrow recovery. More generally, our studies established novel experimental approach to the study of heterologous cell interactions and demonstrated - to our knowledge, for the first time - that single cell RNA-Seq assay can be used as a discovery tool for novel mediators of inter-cellular communication.

Endothelial Cells/Hemangioblasts

T-1191

HIGHLY EFFICIENT AND SCALABLE GENERATION OF FUNCTIONAL ENDOTHELIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS BY A NOVEL GSK3 INHIBITOR.

Christoph Patsch, Martin Graf, Roberto Iacone
Pharma Research Early Development, Roche, Basel, Switzerland

The use of human pluripotent stem cells (PSCs) for *in vitro* disease-modeling and clinical applications is limited by the ability to rapidly produce pure populations of various cell types in sufficient quantities.
Herein, we describe a scalable monolayer protocol to induce vascular cells in chemically defined conditions driven by a novel and potent GSK3ß inhibitor. Within six days we generated large cell populations that are highly enriched for endothelial cells (VE-Cadherin$^+$ ≥85%). We isolated VE-cadherin$^+$ cells by magnetic activated cell sorting (MACS) to ensure pure and homogenous endothelial cell cultures (≥98%). Time-resolved whole-genome expression and selective qRT-PCR analysis revealed a gene expression pattern closely resembling early embryonic vasculogenesis. Further characterizations of VE-Cadherin$^+$ cells, confirm a functional endothelial phenotype. Stem cell-derived endothelial cells give rise to continuous endothelium with dynamic barrier function properties, form vascular network-like structures in angiogenesis assays, show *in vivo* angiogenic potential, convert into activated endothelium after treatment with pro-inflammatory cytokines, thereby facilitating the recruitment of leukocytes. Overall the purified VE-Cadherin$^+$ cells present an endothelial-specific expression pattern; positive for PECAM-1$^+$, CD34$^+$, VE-Cadherin$^+$, vWF$^+$, CXCR4$^+$, VEGFR2$^+$, VEGFR3$^+$ and negative for the hematopoetic lineage markers CD45$^-$, CD43$^-$ and for the smooth muscle cell markers PDGFRβ$^-$, SMA$^-$ The endothelial cell population maintained their cellular identity over the period of cultivation.

This novel and robust method allowed us to reproducibly generated large numbers of homogenous endothelial cells from more than 20 different PSC lines including disease-specific lines from patients that have vascular complication associated with Type 2 Diabetes. With the growing need for defined protocols, our differentiation system may become the standard for deriving endothelial cells at relevant scales appropriate for drug discovery campaigns and regenerative therapies.

T-1192

CHEMICALLY DEFINED OPTIMIZATION FOR EFFICIENT DERIVATION OF HUMAN AND MOUSE ENDOTHELIAL CELLS

William S. Turner, Drew E. Glaser, Andrew B. Burns, Kara E. McCloskey
Engineering, University of California, Merced, Merced, CA, United States

Both mouse and human embryonic stem cells (ESC) can be differentiated into functional endothelial cells (EC) *in vitro*, using either embryoid body formation - which yields low numbers (<1%) of EC - or monolayer cultures. Our monolayer differentiation is performed using a step-wise process incorporating endothelial specific signals from both extracellular matrix (ECM; fibronectin, collagen-type IV, or gelatin) and soluble factors (VEGF, BMP-4, and bFGF). Because fetal bovine serum (FBS) contains a number of unknown soluble factors, making reproducibility challenging and directed differentiation impossible, we examine the defined stage-specific culture conditions, without FBS, for optimization of EC fate from individual ESC human lines (H7 and H9) and mouse (commercially available: E14 and R1, as well as, our own ESC lines: A3 and B2). Our methods optimized the expression of Flk-1/KDR VEGF receptor in the first state (Stage I) of induction and VE-cadherin in the second stage (Stage 2) varying initial seeding density, matrix substrate, and VEGF concentration (Stage I) and all factors including bFGF for Stage II. The results indicate maximal numbers of KDR positive cells on days 14 and 12, for human H7 and H9 ESC lines, respectively, whereas, the four mouse ESC lines expressed maximum numbers of Flk-1 positive cells between 2 and 4 days. Although the matrix and VEGF signaling varied slightly between cell lines, the most significant variable for increasing the number of Flk-1/KDR+ cells was high initial seeding densities. Our PCR data suggests that this cell-cell signaling mechanism may be tied to the Notch signaling pathway. We also found that the ECM signaling for directing EC fate is conserved between murine and human; however, the kinetics is distinct between the two species. For Stage II, high numbers of VE-cadherin positive cells are not observed until two weeks after the Flk-1+ sort for mouse ESC and almost 2 months after KDR+ sort for human ESC, indicating that the maturation of EC in monolayer cultures is much longer than previously published. Our data suggest that fibronectin, not collagen IV, is the best substrata to generate EC. Each cell line, human and mouse have varied optimal amounts of VEGF (0-50ng/ml) and bFGF(0-50ng/ml), most likely due to intrinsic production or the cytokines or naturally high amounts of target receptors. These optimization studies generated serum-free/chemically defined stage- and cell line-specific protocols for the efficient production of EC (over 50%) which can be used for various cell-based therapies, drug screening, as well as to further explore the signaling in EC fate.

T-1193

CO-CULTURES OF ENDOTHELIAL PROGENITOR CELLS AND MESENCHYMAL STEM CELLS FOR THE GENERATION OF VASCULARISED TISSUE ENGINEERED GRAFTS

Mark Chong[1], Dedy Sandikin[1], Renyi Teo[2], Wenhao Leow[2], Junwei Goh[2], Toon Tien Foo[2], Mahesh Choolani[1], Jerry K Y Chan[3]
[1]Obstetrics and Gynaecology, National Univ of Singapore, Singapore, Singapore, [2]Centre for Biomedical and Life Sciences, Singapore Polytechnic, Singapore, Singapore, [3]Reproductive Medicine, KK Women's and Children's Hospital, Singapore, Singapore

Engineered bone tissues are currently limited by inadequate vascularisation in vivo following implantation. Recent research has turned to the use of angiogenic cell sources, including endothelial progenitor cells (EPCs) to generate pre-vascularised tissue prior to implantation. In this study, co-cultures of umbilical cord-blood derived EPCs and

fetal bone marrow mesenchymal stem cells (MSCs) were studied for use in the pre-vascularisation of tissue engineered constructs. Cells were fluorescently-labelled to facilitate imaging and identification in co-cultures. Culture conditions were then optimised in monolayer cultures, and subsequently extended to three-dimensional cultures for applications in tissue engineering. In monolayer cultures, time-lapsed observations demonstrate the cocultures to generate networks, following endothelial cell aggregation and angiogenic sprouting, in a process akin to that of physiological vaculogenesis. Cultures of EPC in MSC-conditioned media failed to elicit similar results, suggesting the need for direct cell contact and the stromal/supportive role of MSC in the system. A modified image analysis method was then developed to characterise vasculogenic events, and used in the optimisation of culture conditions to elicit maximal pre-vascularisation. It was established that culture in complete endothelial growth medium extensive prevascular networks (1.5-fold increase in tube length over conventional culture conditions, $p<0.01$). In addition, MSC were critical for the provision of stromal support, extending vascular longevity in a dose-dependent manner. Extending these results to three-dimensional conditions relevant for tissue engineering, the EPC:MSC co-cultures were induced to form spheroids by culture on non-adhesive plates. Spheroids measuring 500 um in diameter were generated with well-established endothelial networks, which were sustained for up to 21 days of culture. Scaling up to physiologically relevant conditions, co-cultured spheroids were loaded into tissue engineering scaffolds and maintained in a bioreactor for 3 weeks. Outgrowth of cells from spheroids onto the scaffolds was observed and pre-vascularised networks were found to be present, as opposed to direct cell seeding methods. In conclusion, a method to generate pre-vascularised tissue engineered constructs was developed and shown to have several promising features. Work in progress includes evaluation of the constructs in a murine model.

T-1194

HIGHLY ANGIOGENIC CXCR4+CD31+ MONOCYTE SUBSET DERIVED FROM 3D CULTURE OF HUMAN PERIPHERAL BLOOD

Jae-Il Choi, Jin Hur, Ji-Yeon Yun, Jin-A Kang, Seung-Bum Ko, Sang Eun Lee, Han-Mo Yang, Sahmin Lee, Hyun-Jai Cho, Ji-Hoon Hong, Young-Bae Park, Hyo-Soo Kim
Seoul National University, Seoul, Korea, Republic of

Ex vivo expansion of human circulating angiogenic cells is a major challenge in autologous cell therapy for ischemic diseases. Recently, we reported a three-dimensional (3D) culture using human blood cells named as hematospheres which were ideal niches for hematopoietic stem cells (HSCs). Here, we demonstrate that hematosphere-derived CXCR4+CD31+ myeloid cells using peripheral blood possess robust proangiogenic capacity such as formation of vessel-like structures and tip cell-like morphology in Matrigel. We also found that CD31 positive myeloid cells are principal cellular component of hematospheres by magnetic cell sorting. Flow cytometry analysis showed that fresh peripheral blood contained 40.3 ± 15.2 % of CXCR4+CD31+ myeloid cells, but at day 5 of hematosphere culture, most of myeloid cells were CXCR4+CD31+ by 86.9 ± 5.4 %. Hematosphere culture significantly increased the production of angiogenic niche-supporting cytokines. Moreover, CD31-homophilic interaction and VEGF-VEGF receptor loop signaling were essential for sphere formation and acquisition of angiogenic capacity in hematospheres. Matrigel plug and ischemic hindlimb model provide in vivo evidence that hematosphere-derived myeloid cells have highly vasculogenic capacities, participate in new and mature vessel formation, and exert therapeutic effects on ischemic hindlimb. In conclusion, our strategy for ex vivo expansion of human CXCR4+CD31+ angiogenic cells using hematospheres provides an autologous therapeutic cell source for ischemic diseases and a new model for investigating the microenvironment of angiogenesis.

T-1195

A MULTIWELL FLOW CHAMBER SYSTEM TO SCREEN THE EFFECTS OF SURFACE-CONJUGATED PEPTIDES ON ENDOTHELIAL PROGENITOR CELL FATE

Corinne A. Hoesli[1], Catherine Tremblay[2], Pierre-Marc Juneau[2], Bruno Gaillet[2], Carl Duchesne[2], Jean Ruel[2], Alain Garnier[2], Gaétan Laroche[1]

[1]*Université Laval and Centre de Recherche du Centre Hospitalier Universitaire de Québec, Québec, QC, Canada,* [2]*Université Laval, Québec, QC, Canada*

Cardiovascular disease is the leading cause of mortality worldwide. Half of the small-diameter prostheses used in vascular surgeries become occluded within 5 years, which could be prevented if circulating endothelial progenitor cells colonized the graft surfaces. We have previously demonstrated that surfaces functionalized with the fibronectin-derived peptides GRGDS and WQPPRARI promote human saphenous vein cell (HSVEC) adhesion, spreading and expansion. Although mature endothelial cells are used as an initial model for graft endothelialization, the endothelial cells found on vascular grafts *in vivo* originate from bone marrow-derived endothelial progenitor cells. The aim of this work was to develop a live cell imaging system to study endothelial progenitor cell adhesion and differentiation on surfaces functionalized with bioactive peptides in the presence of physiological wall shear stress. A live cell imaging system consisting of 4 parallel-plate flow chambers with separate flow paths to each chamber and a broad wall shear stress operating range (0.5 to 25 dyn/cm^2) was designed. The system was validated by observing the alignment of HSVECs in the direction of flow using time-lapse phase contrast imaging. Fluorophore-tagged RGD micropatterns revealed that endothelial cells preferentially extended pseudopods on the RGD-rich micropatterns during cell adhesion. However, the micropatterned surfaces led to similar HSVEC retention rates of 88 ± 3% after 6 hours of exposure to 15 dyn/cm^2 wall shear stress than surfaces treated with the RGD or WQPPRARI peptide alone. Using this flow chamber system, adherent and circulating cells can be monitored by phase contrast and fluorescence microscopy while applying 4 different test media or shear stress conditions. In addition, a baculoviral reporter system is being developed to observe the differentiation of human umbilical cord blood-derived progenitor cells into mature endothelial cells in real-time. The multiwell flow chamber system is a versatile live cell imaging tool to study the effects of biomaterials, shear stress and soluble molecules on stem cell fate.

T-1196

ROBUST AND EFFICENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO ENDOTHELIAL CELLS USING A CHEMICALLY DEFINED PROTOCOL

Ludivine Challet Meylan, Kiran Musunuru, Chad A. Cowan
HSCRB, Harvard University, Cambridge, MA, United States

Current protocols to differentiate pluripotent cells into endothelial cells (ECs) often rely on embryoid body formation and the use of fetal calf serum. As a result, the differentiation of ECs from human pluripotent stem cells (hPSCs) remains inconsistent, inefficient, and time consuming. Developing differentiation protocols that accurately recapitulating key events regulating cell lineage specification in the embryo has proven to be a powerful and reproducible approach for generating highly enriched populations of differentiated cells. In mammals, endothelial progenitors emerge from lateral and posterior mesoderm in a Wnt dependent fashion that also requires a careful balance of BMP4 and Nodal signaling. Based on these observations, we have developed a novel, rapid, robust and highly efficient protocol for the differentiation of ECs from hPSCs that utilizes the constitutive activation of Wnt signalling via a GSK-3 inhibitor (CHIR-99021). Our protocol results in the differentiation of hPSCs to ECs at efficiencies of 50%-80% in only 6 days. ECs can be further enriched to more than 95% purity by magnetic-activated cell sorting (MACS). Using transcriptional profiles, we determined that these ECs were similar to primary cultures of human ECs (HCAEC and HUVECs) and exhibited mature functional properties including, patent tube formation wound healing, and angiogenic sprouting. We have further tested these differentiated ECs for a number of important metabolic properties such as their ability to transport fatty acids and glucose. Finally, we have used tandem mass spectroscopy to globally profile the metabolic and lipid contents of these ECs and find they are remarkably similar to primary human ECs. These results indicate that the cells could be used to faithfully model human diseases such as obesity, diabetes and cardiovascular disease.

T-1197

DIRECT CONVERSION OF FIBROBLAST TO FUNCTIONAL VASCULAR PROGENITOR CELLS WITH TWO FACTORS

Soo Yong Park[1], Myung Rae Park[1], Yang Woo Kwon[2], Hyun Ah Lee[1], Seung Hun Lee[1], Man Sze Wong[1], Jae Ho Kim[2], Jeong Beom Kim[1]
[1]*Max Planck Partner Group Molecular Biomedicine Laboratory, Hans Schoeler Stem Cell Research Center, Ulsan National Institute of Science and Technology, Ulsan, Korea, Republic of,* [2]*Department of Physiology, School of Medicine, Pusan National University, Yangsan, Korea, Republic of*

Vascular progenitor cell (VPC) is capable of differentiation into both endothelial cells (EC) and smooth muscle cells (SMC). Previous studies have demonstrated the combined transplantation of EC and SMC are synergistically working for blood flow recovery in ischemic hindlimb model.
Here, we generated induced VPC (iVPC) with only two transcription factors using direct conversion technology which exclude from tumorigenicity of induced pluripotent stem cells and embryonic stem cell application.
iVPC spontaneously differentiated into EC and SMC. These two types of cells expressed their lineage specific markers in gene and protein level. VPC-derived EC showed LDL uptake and vascular tube formation in vitro. Functionally, the blood flow recovery and capillary density in ischemic hindlimb were significantly improved in combined VPC-derived EC and VPC-derived SMC transplanted mice.
Our findings suggest that only two factors are sufficient to generate iVPC capable of differentiation into functional EC and SMC can be used as a promising strategy for treatment of ischemic pathologies.

T-1198

USE OF HUMAN PLURIPOTENT STEM CELLS TO DERIVE AND QUANTIFY ENDOTHELIAL CELL-SMOOTH MUSCLE CELL INTERACTIONS IN VITRO

Jason A. Motl[1], Petr Matula[2], Mike A. Lepley[1], Li Li[1], Dan S. Kaufman[1]
[1]*Department of Medicine, University of Minnesota, Minneapolis, MN, United States,* [2]*Faculty of Informatics, Masaryk University, Brno, Czech Republic*

Regulation of angiogenesis is a critical process in normal biological development and in the growth of solid tumors. A number of studies have identified many important endothelial cell (EC) markers and signal transduction pathways essential to this process, but these studies have largely ignored the critical role of support cells during angiogenesis and vasculogenesis. In this novel study we are using human pluripotent stem cell (hPSC)-derived ECs and smooth muscle cells (SMCs) in an *in vitro* assay to identify cell surface markers and signal transduction pathways essential to EC-SMC interactions during angiogenesis. Here, we utilize human embryonic stem cells (hESCs) that stably express either GFP (green) or mCherry (red) or unlabeled

human induced pluripotent stem cells (hiPSCs). We then derive ECs from GFP^+ hESC and SMCs from $mCherry^+$ hESCs or both populations from iPSCs. Prior to the tubulogenesis assay, the iPSC-derived ECs and SMCs are also labeled green or red using CellTracker™ fluorescent probes, respectively. This system allows us to determine computationally the amount of EC and SMC interaction when different small molecules or antibodies are used to up or down regulate angiogenesis or vasculogenesis. Using phase, green and red images, we are have created programs in ImageJ (Fiji) and MATLAB that can identify tubules in an unbiased manner and determine the intensity of the green pixels (ECs) and red pixels (SMCs) as a proxy for EC-SMC interactions in each tubule. We have used this system to test the effect of the PI3K inhibitor, LY294002, in combination with cyclic AMP agonist, 8-bromo cAMP, on EC-SMC interactions and vessel formation. Previous studies in our lab has shown that this treatment upregulates the expression of the arterial endothelial cell marker CXCR4 on ECs. However, preliminary data indicates that this cell surface marker does not appear to play a significant role in changing the interaction between ECs and SMCs (EC to SMC ratio is 1.0556413 in control samples versus 1.0922112 in treated samples), but does change the average number of tubes and average tube length (Total tube number is 557 tubules in control samples versus 690.5 tubules in treated samples; Average tube length is 0.125 in control samples versus 0.101 in treated samples). It appears that inducing the ECs to a more arterial identity increases the role ECs play in tubule formation by creating a greater number of shorter, squatter tubules. We have identified a number of other compounds that affect the identity of ECs, which we will test in this quantitative co-culture assay of vasculogenesis. This assay allows for the novel application of hPSC-derived cells in an *in vitro* angiogenesis assay to understand vascular interactions and identify additional therapeutic targets for both cancer and revascularization therapies.

T-1201

SENESCENCE OF HUMAN ENDOTHELIAL PROGENITOR CELLS IMPAIRS THEIR VASCULAR REPAIR CAPACITY

Reinhold Medina[1], Christina L. O'Neill[1], Sarah Chambers[1], Jasenka Guduric-Fuchs[1], Emma Reid[1], Jessica Neisen[2], David Waugh[2], David Simpson[1], Alan Stitt[1]

[1]Centre for Vision & Vascular Science. Queen's Univ Belfast, Belfast, United Kingdom, [2]CCRCB. Queen's Univ Belfast, Belfast, United Kingdom

Endothelial progenitor cells (EPCs) have been tested as an alternative therapy to promote vascular regeneration in ischemic pathologies such as myocardial infarction, stroke, peripheral vascular disease, and ischemic retinopathies. Obtaining sufficient numbers of EPCs for autologous therapy may become an obstacle for the feasibility of this treatment, because in vitro expansion methodologies for adult stem/progenitor cells ultimately lead to replicative senescence. Here, we examined the senescence programme in human EPCs cultured in vitro, determined if this impairs their vasoreparative properties, and identified responsible molecular mechanisms.
EPCs isolated from human peripheral and umbilical cord blood were expanded in culture until they reached their Hayflick limit. The senescence phenotype was characterised by growth curves, cell cycle analysis, senescence-associated beta-galactosidase evaluation and DNA damage quantification. Furthermore, changes in gene/protein expression associated with EPC senescence were assessed by transcriptome, proteome and secretome analysis. EPC function was evaluated in vitro by migration and tubulogenesis assays. The therapeutic potential of senescent EPCs was evaluated in vivo using the mouse oxygen-induced retinopathy (OIR) model.
Late passage EPC proliferation capacity diminished significantly, with more than 70% of cells arrested in G0/G1 phase of the cell cycle. These late passage EPCs displayed a significant increase in cytoplasmic volume, increased β-galactosidase activity and accumulated γ-H2AX foci ($p<0.001$). There was also a significant decrease in telomerase activity coupled with telomere shortening. Senescent (late-passage) EPCs demonstrated impaired migratory and tubulogenic capacity compared to early-passage EPCs ($p<0.001$). Integrated transcriptome-proteome analysis identified inflammatory signalling pathways as major components of the EPC senescence programme, with IL8 identified as an important senescence facilitator. shRNA mediated knock-down of IL8 in EPCs significantly extended ex-vivo lifespan, delayed senescence and enhanced cellular function. In OIR, both early and late-passage EPCs significantly reduced retinal avascular area compared to vehicle treated eyes ($p<0.001$). However, senescent EPCs showed

reduced ability to integrate into the retinal vasculature compared to early-passage EPCs. Furthermore, only early-passage EPCs significantly reduced the area of pathological pre-retinal neovascularisation ($p<0.001$).
Ex-vivo expansion of human EPCs ultimately leads to replicative senescence linked to cellular dysfunction in vitro and in vivo. Modulation of EPC senescence is an important strategy to improve the potential for their usage as an autologous cell therapy.

T-1202

IMPROVING CELL FUNCTIONS AND THE ENRICHMENT OF LATE OUTGROWTH ENDOTHELIAL PROGENITOR CELL COLONIES USING A GLYCOSAMINOGLYCAN (GAG) MIMETIC

Fabien CHEVALIER[1], Melanie LAVERGNE[2], Segolene FERRATGE[2], Fernando SINERIZ[3], Georges UZAN[2], Patricia ALBANESE[1]
[1]Laboratoire CRRET, University of Paris Est Creteil, CRETEIL, France, [2]INSERM U972, PARIS, France, [3]OTR3 Company, PARIS, France

Clinical trials based on cell therapy in the field of cardiovascular diseases have been conducted and have shown either negative effects or none at all. Even some marginal effects are observed, they are probably due to a paracrine effect of injected cells secreting growth factors, which die few days after intervention. Otherwise, inflammatory processes triggered by tissue degeneration lead to the destruction of extracellular matrix, and to an important tissue remodelling resulting in loss of functionality. Our hypothesis is that the quality of the microenvironment in which therapeutic cells are injected is a key point in biotherapies success. We focus our interest on heparan sulfates (HS) and chondroïtin sulfates (CS), the polysaccharides associated to core protein to form proteoglycans. These GAGs are important for protecting and stimulating activity of heparin binding growth factors. Otherwise, endothelial progenitor cells (EPC) are ideal candidates for the treatment of ischemic tissues. Our main objective is to develop an innovative strategy of biotherapy after ischemic events, combining a well-defined ex vivo amplification procedure preserving EPC physiological potential, and a matrix therapeutic product based on GAGs mimetics. The colony forming efficiency of EPC is a very critical step in production of therapeutic endothelial cells, as it will determine the first pool of endothelial-colony forming cell (ECFC) in vitro. The capacity of one GAG mimetic to improve this step was evaluated on EPC from human cord blood mononucleated cells: addition of the mimetic in medium during isolation steps leads to an enrichment of 2 to 3 times more ECFC. This promising result leads us to further analyze EPC properties. Here, we compared the effect of Heparin, as a natural GAG, and the GAG mimetic in vitro. The two compounds are able to promote cell proliferation and migration, by potentiating growth factors activities. However, only the GAG mimetic is able to increase EPC adhesion and self-renewal abilities. Published data had already demonstrated matricial effects of injected GAGs mimetics after injury, that are able to reorganize the microenvironment leading to a better tissue regeneration. Finally, our promising results on EPC properties highlight the importance of GAGs as cellular regulator for the development of new therapeutic protocols: (1) they optimize the critical parameters of in vitro EPC-colony formation and amplification to obtain a sufficient amount of cells for a therapeutic application, and (2) they will improve cell properties required after in vivo injection such as cell adhesion, migration and self-renewal.

T-1203

A NOVEL SOURCE OF ENDOTHELIAL PROGENITOR CELLS FOR VASCULAR REGENERATION

Mairi Brittan, Amanda Hunter, Elizabeth M. Skinner, Susan Gallogly, Nicholas Mills
Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, United Kingdom

Background. Endothelial progenitor cells (EPC) circulate in adult human blood and contribute to neovasculogenesis in mice and therefore represent a potential source of cells for vascular regenerative therapies. EPC are isolated following in vitro growth of peripheral blood mononuclear cells, but are present in very low frequencies in the circulation and therefore alternative strategies for harvesting EPC for clinical applications are urgently required. We have optimised a method for extracting “vascular EPC” from the venous intimal layer using a minimally invasive

wire biopsy technique. We herein propose a novel method for expanding vascular EPC for therapeutic applications. We have characterised and compared the phenotype and function of vascular EPC compared to EPC isolated from blood mononuclear cells (circulating EPC) from the same subjects. Methods. Subjects (n=7) attended the Clinical Research Facility, Royal Infirmary of Edinburgh. Under local anaesthetic, a J-shaped guidewire was inserted via a venous sheath into a superficial forearm vein and gently manipulated to harvest cells. The wire was then inserted into sterile tubing and media syringed through the tubing to dislodge cells. Cells were collected by centrifugation and cultured in endothelial cell growth media. Circulating EPC, were isolated from peripheral blood mononuclear cells by gradient centrifugation and maintained in identical conditions to vascular EPC. Growth kinetics of circulating and vascular EPC were compared and expression of panels of endothelial- and stem cell-specific antigens was determined at intervals by polychromatic flow cytometry. An established in vitro assay of angiogenesis was used to compare endothelial function by cellular progeny of vascular and circulating EPC. Results. Vascular cells isolated by wire biopsy formed highly proliferative endothelial outgrowth colonies. Cell progeny of vascular and circulating EPC displayed a similar morphology, endothelial cell antigen expression (CD31, VE-cadherin, KDR, Tie2, CD146, vonWillebrand factor, eNOS), and angiogenic potential. However, vascular EPC displayed increased expression of the stem cell antigen CD34, produced greater numbers of outgrowth colonies (mean±SD total colony number (n=7): vascular EPC=18.3±6.3, circulating EPC=6.3±6.1; p=0.06 student's t-test) and were viable for longer in culture compared to circulating EPC. Conclusion. Wire biopsy represents a novel means of harvesting EPC from the venous wall with an enhanced efficiency and yield compared to existing methods. Vascular EPC have several advantages over circulating EPC; augmented clonogenicity, increased endothelial progeny and prolonged lifespan. Therefore, vascular EPC show great potential for future clinical application in the development of novel vascular regenerative therapies.

T-1204

THE IMPORTANCE OF CELL SOURCE FOR SOMATIC TISSUE REPROGRAMMING: ENDOTHELIAL CELL-DERIVED IPS CELLS HAVE ENHANCED CAPACITY TO DIFFERENTIATE INTO FUNCTIONAL ENDOTHELIAL CELLS

Elizabeth Skinner[1], Mairi Brittan[1], Olga Tura[1], Judy Fletcher[1], Peter Burton[2], Andrew Baker[2], Joanne C. Mountford[2], Patrick Hadoke[1], Ian Wilmut[1], Nicholas L. Mills[1]

[1]University of Edinburgh, Edinburgh, United Kingdom, [2]University of Glasgow, Glasgow, United Kingdom

Background: Induced pluripotent stem (iPS) cell technology has primarily focused on the reprogramming of fibroblasts to an embryonic stem (ES)-like state. However, as the field has developed, the importance of cell source has been studied to enhance reprogramming and differentiation efficiencies.
Objectives: To generate iPS cells from human fibroblasts and endothelial outgrowth cells from blood. To compare reprogramming efficiency between both cell types, and compare their potential for endothelial differentiation.
Methods and Results: Reprogramming: Episomal vectors containing Sox2, Klf4, Oct4 and c-Myc were electroporated into fibroblasts and endothelial cells using the Amaxa system. Successfully reprogrammed fibroblast-derived iPS ('fiPS') and endothelial cell-derived iPS ('eiPS') arose as colonies, and were picked and expanded. Reprogrammed cells expressed pluripotency markers SSEA3, SSEA4, TRA-1-60, Oct4 and NANOG, and developed into all three germ layers following embryoid body (EB) formation.
Optimisation of endothelial differentiation protocol: iPS and ES cell lines were aggregated into EBs for three days in stem cell growth media containing mesoderm-inducing cytokines. EBs were then disaggregated and cultured in Endothelial Growth Medium supplemented with VEGF. After seven days, a population of CD31+ cells was isolated by FACS sorting, cultured and mature endothelial cell antigen expression determined using flow cytometry. CD31+ cells were selected for functional assessment in vitro using established assays of angiogenesis, migration and adhesion. Human endothelial cells derived from iPS cells were implanted subcutaneously in a NOD-SCID mouse model of angiogenesis and neovasculogenesis quantified at day 21.
Comparison of fiPS and eiPS: eiPS differentiate into endothelial cells with greater efficiency than fiPS (15.2% and 4.1%, respectively). fiPS-endothelial cells have been characterized phenotypically and shown to express endothelial markers CD146 (86.2% ± 10.1%), CD31 (92.9% ± 3.3%), VEFGR2 (44.8% ± 2.8%), Tie-2 (30.7% ± 19%) and VE-Cadherin (65.0% ± 7.1%). When grown on Matrigel ™, they form tubule-like structures with a similar number of vessel

connections per field to control endothelial cells (54.5 ± 5.5 versus 57.5 ± 4.0). Characterisation of eiPS-endothelial cells is ongoing. In vivo, implantation of endothelial cells derived from fiPS and eiPS increase vessel formation by 78.2% ± 16.4% and 67.2% ± 7.6% respectively, compared to Matrigel ™ control. By comparison control endothelial cells increased vessel formation by 6.9% ± 0.8%.
Conclusion: Endothelial cells can be isolated from blood and reprogrammed effectively to form eiPS cell lines with greater capacity to differentiate into endothelial cells than iPS cells derived from dermal fibroblasts suggesting reprogrammed cells retain epigenetic memory. Endothelial cells derived from both eiPS and fiPS cells increase angiogenesis compared to mature endothelial cells and have potential therapeutic applications for vascular regeneration.

Other

T-2011

HYPOXIA-MIMICKING AGENTS PROMOTE CXCR4 GENE EXPRESSION AND MIGRATION OF MESENCHYMAL STEM CELLS TOWARDS SDF1

Hamid Reza Bidkhori[1], Ahmad Reza Bahrami[3], Hojjat Naderi-Meshkin[4], Asieh Heirani[4], Mahdi Mirahmadi[4], Mina Shahryari[4], Nasser Sanjar Moussavi[5], Naghmeh Ahmadiankia[6], Malihe Hassanzadeh[4], Moein Farshchian[7], Maryam M. Matin[2]
[A]Department of Biology, Faculty of Science, [B]Department of Biology, [1]Ferdowsi University of Mashhad, Mashhad, Iran, Islamic Republic of, [2]Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran, Islamic Republic of, [3]Stem Cells and Regenerative Medicine Research Department, ACECR-Mashhad Branch, Mashhad, Iran, Islamic Republic of, [4]Department of surgery, Facaulty of medicine, Islamic azad university-Mashhad branch, Mashhad, Iran, Islamic Republic of, [5]Shahroud University of Medical Sciences, Shahroud, Iran, Islamic Republic of, [6]Department of Biology, Ferdowsi University of Mashhad, Mashhad, Iran, Islamic Republic of

Despite that exploration of potential clinical sources of mesenchymal stem cells (MSCs) is giving rise into great promise in the field of regenerative medicine, proper homing is still a prerequisite to exhibit any type of activity in the target tissue. CXCR4 is among the very well known molecular components involved in cell invasion and migration. The culture-expanded MSCs almost completely lose their engraftment potential without this chemokine receptor. However their homing properties can be enhanced through chemical pre-treatments that would increase CXCR4 expression even after few passages.
To the best of our knowledge, there is no comparison between bone marrow and adipose tissue derived MSCs subjected to various hypoxia-mimicking agents. To find the best pretreatment condition for increasing the CXCR4 expression and subsequently enhancing the migration of MSCs towards SDF1, in this study, we explored the effects of different hypoxia-mimicking agent such as valproic acid (VPA), CoCl2, desferrioxamine (DFX) and Licl2 on CXCR4 expression in MSCs and their migration under normoxic condition.
To this end, MSCs derived from adipose tissue and bone marrow were subjected to these pretreatments, CXCR4 expression was evaluated by real-time RT-PCR, and *in vitro* migration of the MSCs was investigated comparatively by TransWell migration assay towards SDF1. Results showed different expression patterns of CXCR4 induction and different migration potentials not only among various hypoxia-mimicking agents but also between two sources of the MSCs. The best result was shown by pretreatment of adipose tissue-derived MSCs by DFX.

T-2012

PREVENTIVE AND RESTORATIVE EFFECTS OF UMBILICAL CORD BLOOD (UCB) MONONUCLEAR CELLS (MNCS) IN ACUTE KIDNEY INJURY (AKI) INDUCED BY CYCLOSPORIN A (CSA), IN RATS.

Ana Paula Coelho Gutierres[1], Fernanda Teixeira Borges[1], Manuel Jesus Simões[2], Nestor Schor[1]

[A]Nephrology Division, Department of Medicine, [B]Department of Morphology, [1]Escola Paulista de Medicina/ UNIFESP, São Paulo, Brazil

CsA has been given great medical advances, especially in solid organ transplantation, but the main problem still is nephrotoxicity. In order to avoid or minimize the AKI induced by CsA, it was obtained cells from UCB and evaluated in CsA nephrotoxic AKI model. MNCs were obtained between gestation day 19th and 20th from Wistar rats. In order to induced AKI, rats were submitted to a low salt diet (7 days before and during all studies) and given CsA or Olive Oil (20 mg/kg, ip) for 14 days. In the 7th day of the treatments, MNCs were administered (1x106 cells per day)during 7 days together with CsA or Olive Oil. It was evaluated creatinine, urea, sodium and potassium as well as morphological alterations. No differences were observed by morphological analysis at the 7th day of the treatment, since it was observed acute tubular necrosis with or without MNCs. It was also observed a significant interstitial fibrosis. Although the administration of MNCs constitute a myriad of stem cells (progenitor, hematopoietic, endothelial, mesenchymal) they are not able to modify the CsA nephrotoxicity during this period of 7 days.Differently, in the animals receiving treatment for 4 days with MNCs or Mesenchymal Stem Cells (MSC), it was observed a significant improvement in creatinine as well as in the morphology suggesting that in this time, the nephrotoxicity is still reversible. According to the biochemical values associated with morphological analysis, it was observed that MNCs derived from Wistar UCB rats have effects repairing and / or shield the AKI caused by CsA but only in the early injury period, indicating that as soon is suspected of AKI, the MNCs could be administered in order to prevent further kidney damage.

T-2013

DEVELOPMENT OF NEONATAL MOUSE INJURY MODELS TO ASSESS HUMAN VERY SMALL EMBRYONIC LIKE STEM CELL REPAIR AND REGENERATION

Jiusong Sun, Subhabrata Biswas, Quan Zhu, Wayne A. Marasco
Dana-Farber Cancer Institute, Boston, MA, United States

Studies of human stem cell function in the repair of damaged, diseased and degenerative tissues have traditionally been limited to *ex vivo* analyses and non-invasive procedures. Clinical trials are costly and severely limited due to ethical constraints. Reliable small animal models of tissue injury that could be used to evaluate human stem cell-based repair and regeneration would provide scientists with much needed human disease models and more predictive ways to test therapeutics for a variety of diseases. We have demonstrated proof-in-principle of this concept in neonatal mouse models that can undergo spontaneous repair of injured heart, lung and kidney tissue if it occurs within 24 hours of birth. Human very small embryonic like (hVSEL) stem cells are pluripotent stem cells involved in the normal turnover and regeneration of tissues and their circulating levels greatly increase in response to injury. Adult human VSELs are CD133+/ Lin-/CD45- pluripotent cells and are capable of differentiation into cells from all three germ lineages. We have successfully developed three neonatal NSG mouse injury models, including heart injury, lung injury and kidney injury. Using kidney injury model, we found anti-human CD31+ cells accumulation in the injury area with hVSELs injection. It indicated that hVSELs may participate in injury repair and regeneration. Our work provided three injury models to determine the fate and function of the stem cells in normal tissue homeostasis as well as to assess their therapeutic potential for tissue repair and regeneration. It will also accelerate research in the stem cell field by providing humanized mouse models to study human stem cell engraftment and differentiation in regenerative medicine.

T-2014

POTENTIAL USE OF PATIENT SPECIFIC INDUCED-PLURIOPOTENT STEM CELL (IPSC) TO DELINEATE THE MOLECULAR PATHOGENESIS OF SYNDROMIC HIRSCHSPRUNG (HSCR)

Jasmine Yung[1], John Wong[2], Sin-Ting Lau[1], Hung-Fat Tse[3], Paul Tam[1], Maria-Mercedes Garcia-Barcelo[1], **Elly Ngan**[1]
[A]Dept of Surgery, [B]Dept of Psychiatry, [C]Dept of Medicine, [1]Univ of Hong Kong, Hong Kong, Hong Kong

Hirschsprung (HSCR) disease is a complex congenital disorder attributed to a failure of enteric neural crest cells (NCCs) to fully colonize the bowel and form ganglia in the hindgut, leading to bowel obstruction and megacolon. A significant number of HSCR patients also suffer from other disorders of NC origin, such as ventricular and atrial septal defects (VSD/ASD). The pathologies of these disorders are largely unclear. Recently, we have established iPSC lines from a syndromic HSCR patient with VSD/ASD. While these patient iPSCs showed similar capacity for generating NCCs (p75NTR+; HNK1+), their NCC derivatives exhibited severe differentiation defects in making neurons and cardiac smooth muscle cells (SMC). Intriguingly, the neural differentiation defects were restricted to NC lineage. The capacity of patient iPSC to make various types of CNS progenitors (PAX6+) and neurons was comparable to that of the control iPSC, nicely recapitulating the patient's phenotype where only enteric neurons, but not CNS progenitors were affected. These observations prompted us to further delineate the causative genes/pathways that lead to failure of making neurons/glia or cardiac SMCs. By transcriptome sequencing, we revealed that FACS sorted p75NTR+; HNK1+ population express various NC markers (e.g. SNAI1, NGFR(p75NTR), TWIST, TFAP2A, SOX10, PHOX2B, SOX9, SOX10, RET and PAX3), a gene expression pattern conferring multipotency of NCCs. Subsequent comparative analysis of the transcriptomes of the control and patient iPSC-derived NCCs and their derivatives showed that pathway genes implicated in neural (ERBB4, NGFR, EDNRB, SOX10, NGF, EDN1/2) and SMC (e.g. GATA6, HAND1) differentiation are consistently dysregulated in the patient cells. More importantly, premature upregulation of glial differentiation markers was detected in the patient NCCs, suggesting that the neuronal differentiation defects of patient NCCs would be the result of differentiation bias towards glial lineage. Taken together, our data indicated that NCCs generated from patient iPSC could serve as a useful human disease model for a better understanding of molecular pathogenesis of neurocristopathies.

T-2015

EPIDERMAL GROWTH FACTOR REGULATES DE DIFFERENTIATION OF NEURAL CREST CELLS IN QUAIL (COTURNIX JAPONICA)

Andrea G. Trentin, Bianca L. Teixeira, Giordano W. Calloni, Ricardo C. Garcez
Biologia Celular, Embriologia e Genética, Universidade Federal de Santa Catarina, Florianópolis, Brazil

The neural crest (NC) corresponds to a transient structure of vertebrate embryos originated at the neural borders of closing neural tube, during neurulation. The NC contains a mixed population of already defined precursors and multipotent cells that can give rise to a great variety of cell types. Among the derivatives of the NC are neurons and glial cells of the peripheral nervous system, skin melanocytes and mesenchymal derivatives. NC cell fate and differentiation are strongly influenced by microenvironmental factors, such as the epidermal growth factor (EGF), found along migratory paths and in target tissues. We have previously demonstrated that EGF promotes the differentiation of quail trunk NC cells to melanocytes and neurons whereas fibroblast growth factor 2 (FGF2) stimulates the glial differentiation *in vitro*. In addition, we have recently shown the important role of FGF2 in maintaining the stemness of avian NC cells by stimulating multipotent NC progenitors, and promoting the self-renewal of bipotent NC cells endowed with glial and SMC potentials. In the present study, we used both mass cultures and single-cell culture assays to investigate the effects of EGF in the differentiation and fate of quail (*Coturnix japonica*) NC cells at both trunk and cephalic levels. Migrating NC cells express EGF receptor in culture as revealed by immunofluorescense analysis. The results also demonstrate that EGF does not influence the NC cell proliferation rate (assessed by BrdU incorporation) or the mesenchymal differentiation in mass cultures neither the proportion of NC progenitors in single cell culture assays. However, investigating the melanocytic differentiation, we observed that EGF significantly increase the proportion of pigmented melanocytes in relation to the MelEM (melanocyte earlier marker)-positive melanoblasts. Therefore, in this study we show for the first time that EGF regulates the final stages of melanocytic differentiation, possibly the melanin synthesis, rather than NC proliferation or fate. Understanding the role of EGF on melanocytic differentiation will contribute to advances in studies of diseases involving changes in pigmentation pattern, and its consequences in vertebrate organisms.

T-2016

EXTRACTS FROM NEURAL STEM CELLS PROMOTE HAIR GROWTH

Insik Hwang
Lab.of Stem Cell Biology, Department of Biomedical Science, Seoul, Korea, Republic of

Alopecia is the loss of hair, and can be caused by various reasons such as aging, genetics, chemotherapy and malnutrition including damage to the hair shaft or follicles. However, current therapies using finasteride, the dihydrotestosteron-suppressing 5a-reductase inhibitor, and minoxidil, the anti-hypertensive potassium channel opener limit their therapeutic uses due to their undesirable side effects and low efficacy. The dermal papilla cells (DPCs), located at the base of hair follicle and is thought to be fundamental for hair follicle morphogenesis and cycles of growth (anagen phase), regression (catagen phase), and rest (telogen phase). Several factors such as vascular endothelial growth factor (VEGF), insulin-like growth factor-1(IGF-1), hepatocyte growth factor (HGF), have been thought to be important for anagen maintenance. In this study, we showed that the extracts from neural stem cells (NSCs) promoted the hair growth in C57BL/6 mice. The C57/BL-6 mice were treated with the extracts, and the length of hair growth and the weight of shaved hairs were significantly increased compared to control. In addition, the mRNA expression levels of VEGF, IGF-1, and HGF were dramatically increased in dermal papilla cells (hDPCs). Interestingly, sonic hedgehog (Shh) was robustly increased, and transforming growth factor beta 1 (TGF-β1) as a suppressor of hair growth was decreased in hDPCs in our real-time RT-PCR. These results showed that extracts from NSCs induced several growth factors in *in vitro* system using hDPCs and stimulated hair growth in *in vivo* system using mice. Therefore, the extracts could be used as raw materials to prevent the hair loss in the future.

T-2017

UTILIZING FLOW CYTOMETRY, IMMUNOHISTOCHEMISTRY AND MORPHOLOGY TO ASSESS MICROGLIAL ACTIVATION STATES IN A MOUSE MODEL OF TRAUMATIC BRAIN INJURY

Supinder S. Bedi, Philippa Smith, Robert hetz, Henry Caplan, Charles S. Cox
Pediatric Surgery, University of Texas, Houston, TX, United States

Background: Traumatic brain injury (TBI) affects nearly 1.5 million patients in the United States annually, resulting in an annual economic impact of $60 billion. We have previously demonstrated the role of bone marrow derived stem cells to modulate macrophages (microglia)associated with post-TBI neuroinflammation. Early activation of CNS macrophages (microglia) has been observed in TBI; classically activated M1 macrophages may produce pro-inflammatory and neurotoxic substances, while those in an M2 (alternatively activated, anti-inflammatory) state are neuroprotective. Techniques for assessing microglial activation states ex vivo are critical to understanding TBI.
Methods: Normal adult C57B6 mice 6-8 weeks old were subjected to controlled cortical impact (CCI) injury, sham injury or LPS induced inflammation and sacrificed 24h later. Microglia were isolated from brains by enzymatic digestion and mechanical dissociation followed by myelin removal and magnetic bead separation. Enriched cell populations were surface-stained with antibodies against markers CD45 (leukocyte), CD11b (myeloid), FcγRII/III (M1), and CD206 (M2); data was acquired with a BD LSRII cytometer and analyzed with FlowJo. In addition, we used some of the mice for immunohistochemistry to assess microglia (IBA1) M1 (CD86) and M2 (CD206) states in conjunction with morphological activation and resting states.
Results: CD11b+ microglial cells were reliably enriched (>75%). In all brains, two CD11b+ populations differing in forward scatter were obtained, with the ratio of FSClo/FSChi cells increasing slightly in injured brains. Most (>90%) microglia in all brains were CD45lo, but a population of CD45hi cells associated with the higher FSC population increased from 4% in shams to 7.5% in injured brains. Both percent positive and fluorescence intensity of M1 marker FcγRII/III increased in injured microglial populations while M2 marker CD206 expression decreased. There was evidence of activated microglia in the CCI and LPS-injected mice in comparison to sham. There was an absence of CD 206 in the sham animals.
Conclusions: We have developed a flow-based method for evaluating microglial activation states ex vivo. In addition, we have used immunohistochemistry and morphological assessment to further classify activation states of microglia.

T-2018

BASE EXCISION REPAIR INDUCED GENOME DESTABILIZATION: CAUSE OR CONSEQUENCE OF HUMAN EMBRYONIC STEM CELLS ADAPTATION?

Miriama Kruta[1], Monika Seneklova[2], Jan Raska[2], Lukas Balek[2], Petr Fojtik[2], Petr Dvorak[1], Vladimir Rotrekl[1]
[1]Department of Biology, Faculty of Medicine, Masaryk University and International clinical research center St'.Anne's University Hospital Brno, Brno, Czech Republic, [2]Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Genome stability of in vitro cultivated human embryonic stem cells (hESC) is one of the necessary requirement of their potential beneficial use in regenerative medicine. It is known, that prolonged maintenance of hESC under in vitro conditions leads to adaptation accompanied by growth acceleration and genome destabilization. Adaptation process during prolonged cultivation of hESC enables us to use early and late passage hESC as a model of adaptation to culture.
One of the possibly most eloquent measures of genome instability is frequency of mutations. We have designed simple and robust assay to determine the mutant frequency (MF) in hESC based on the mutagenesis of hypoxanthine phosphoribosyltransferase (HPRT) gene. Our data show that the adaptation process is indeed connected to increased MF suggesting that our HPRT assay can be used as qualitative marker of hESC adaptation.
In a quest to dissect the molecular mechanisms leading to adaptation we have also recently shown that one of the mechanisms failing during adaptation to cultivation conditions is base excision repair (BER) mediated by decrease in level of apurinic/apyrimidinic endonuclease APE1 in late passage hESC. In order to determine if the failing BER/APE1 stands also behind the genome instability in adapted hESC, we have created model by downregulating APE1 in hESC via siRNA as well as inhibiting BER in hESC using methoxamine. Both models presented significant increase in MF in hESC both spontaneous as well as induced by ionizing radiation.
Our results support the concept where adaptation induced failing BER increases MF, which in turn accelerates the process of adaptation closing the vicious cycle of hESC adaptation to culture conditions. At the same time our results demonstrate the robustness of our MF assay, which can be used to evaluate the genome stability of hESC.

T-2021

POINT MUTATIONS IN ES CELLS

Yuko H. Fujimori, Mayumi Sugiura, Yasuji Kasama, Misato Sunayama, Masahiro Uda, Miki Nakamura, Shunsuke Ando, Ryoko Araki, Masumi Abe
Stem Cell Biology & Bioinformatics Team, National Institute of Radiological Sciences, CHIBA, Japan

Whether induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) are identical type of pluripotent stem cells or not, is vital issue for iPSCs biology. Here we attempt to address the issue via focusing on the point mutations in their genomes.
Over 1,000 point mutations have been observed in the genomes of various iPSC lines established from both human and mouse by different ways. On the other hand, it has been shown that substantial numbers of single nucleotide variants (SNVs) existed in a small portion of the parent somatic cells population already. Therefore, currently whether the point mutations observed in an iPSC genome arose at the iPSCs generation or those were pre-existing SNVs, becomes a highly controversial issue. A comparison with ES cell genomes is extremely fruitful for addressing these issues. Nevertheless, point mutations analysis of ES cells genomes has never been achieved until now. The reason why the analysis of point mutations in ES cells has not been feasible is that their parent genomes, which must be used for identifying point mutations as controle, are always not available, especially in human case.
Here, we established ES cell lines from an inbred mouse strain, C57BL/6, along with the genomes of both parents. Using 61 blastocysts, we successfully obtained 16 of ES lines, eight of male and eight of female. Their fully developmental ability was confirmed by aggregation experiments; 100% chimerism and germline competence were verified. On the four ES lines, two of male and two of female, and on their parent genomes we conducted whole genome sequencing to identify point mutations. Our analysis covered approximately 55% of the entire genomes with more than 10 redundancies. In conclusion, we identified from 10 to 30 point mutations in their genomes, and

then confirmed the mutations by Sanger sequencing. Surprisingly, the frequencies were ~1/10 of those observed in iPSCs genomes. In addition to the frequency, we found a difference in their profile between iPSCs and ES cells. Clear difference in the point mutations between ES cells and iPSCs may suggest that most point mutations observed in iPSCs genomes arose associated with their conversion process from somatic cells.

T-2022

CAN AMNION AND AMNIOTIC FLUID BE CONSIDERED A STEM CELL NICHE?

Enrica Bertin[1], Martina Piccoli[1], Chiara Franzin[1], Giovanna Spiro[1], Andrea Alex Schiavo[1], Arben Dedja[2], Paolo De Coppi[3], Michela Pozzobon[1]

[1]Department of Woman and Child's Health, University of Padua, Fondazione Istituto Ricerca Pediatrica Città della Speranza, Padova, Italy, [2]Department of Cardio-Thoracic and Vascular Sciences, University of Padua, Padova, Italy, [3]UCL Institute of Child Health and Great Ormond Street Hospital, London, United Kingdom

Background: The stem cell niche is the microenvironment where stem cells reside. Different elements define the niche and regulate stem cell characteristics, like stromal support cells, gap junctions, soluble factors, extracellular matrix proteins, blood vessels, and neural inputs. Several studies demonstrated the presence of stem cells in human and murine amniotic fluid (AF), both types of cells have been isolated by the selection for the marker c-Kit and are called Amniotic Fluid Stem (AFS) cells. AFS cells are characterized by the expression of both multipotency and pluripotency markers, differentiate in culture into all the three embryonic lineages and can be reprogrammed without any genetic manipulation. However, it is still unclear where AFS cells originate.
Aim: To date, this is the first study investigating the role of amnion and amniotic fluid as stem cell niche of AFS cells.
Methods: Through in utero transplantation (IUT), we injected YFP positive mouse embryonic stem (ES) cells into the AF of E13.5 wild type fetuses (C57BL6/J), and 4 days after IUT we evaluated their characteristics by immunofluorescence and qRT-PCR for the expression of pluripotency markers (Oct4, Sox2, Nanog, c-Myc, Klf4). We also performed cytokines analysis using ELISA assay for stromal derived factor (SDF-1), vascular endothelial growth factor (VEGF), angiopoietin-1 and stem cell factor (SCF), both in the AF before and after mouse ES cells injection; we also performed immunofluorescence analysis for the pluripotency markers in the amnion after IUT.
Results and Conclusion: Mouse ES cells isolated from the AF and the amnion at E17.5 maintained the expression of Oct4, Sox2, c-Myc, Klf4 and Nanog. We were able to demonstrate that AF and amnion are prone to sustain the expression of pluripotency markers and to maintain mouse ES cells in a quiescent state. Cytokine analysis of the AF showed the presence of small cell-signalling molecules characteristics of other stem cells niches such as SCF and SDF-1.
This is the first indication that stem cells may reside in AF and amnion without differentiating, as it occurs in stem cell niches. We can postulate that the interplay between AFS cells and the niche creates the dynamic system necessary for sustaining the undifferentiated state of the cells themselves.

T-2023

DEVELOPMENT OF A HIGH-CONTENT OXIDATIVE STRESS ASSAY IN 1536-WELL MICROTITER PLATE FORMAT FOR HIGH-THROUGHPUT SCREENING (HTS) OF ASTROCYTES DIFFERENTIATED FROM HUMAN EMBRYONIC STEM CELLS

Natasha Thorne[1], Nasir Malik[2], Bradley M. Class[1], Sonia Shah[2], John C. McKew[1], Mahendra S. Rao[3], Wei Zheng[1]

[1]National Center for Advancing Translational Sciences/Therapeutics for Rare and Neglected Diseases, NIH, Rockville, MD, United States, [A]NIAMS/Laboratory of Stem Cell Biology, [B]NIAMS/Laboratory of Stem Cell Biology/NIH Center for Regenerative Medicine, [2]NIH, Bethesda, MD, United States

It is becoming increasingly clear that astrocytes play key roles in proper neuronal function in the central nervous system. Astrocytes serve to maintain an extracellular environment conducive to neuronal signaling, in addition to

supplying neurons with nutrients necessary for cell health and metabolic precursors that can be used for neurotransmitter synthesis. Recently, astrocytes have been implicated in the pathogenesis of a variety of neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis (ALS), and have thus become a new target for drug discovery. In order to identify potential therapeutics for these diseases, we have concentrated our efforts to developing astrocyte-based assays that can be used for high-throughput screening, and that can be used to screen astrocytes differentiated from induced pluripotent stem (iPS) cells from neurodegenerative disease patients. Towards this effort, our group has developed methods for successfully growing and maintaining astrocytes differentiated from human embryonic stem (hES) cells in 1536-well microtiter plate format – a format that can be used for high-throughput screening (HTS). Additionally, we have developed a high-content screening assay in 1536-well format to identify compounds that are cytoprotective to astrocytes challenged with oxidative conditions – conditions characteristic of neurodegenerative disease. This miniaturized oxidative stress assay can be used to screen large chemical libraries using patient-derived astrocytes to identify lead compounds for drug development.

T-2024

GLUTAMINE INCREASES OSTEOGENIC DIFFERENTIATION OF DENTAL FOLLICLE STEM CELLS (DFSCS) BY UPREGULATING BONE MORPHOGENETIC PROTEIN 2 (BMP2) AND BONE MORPHOGENETIC PROTEIN 6 (BMP6) EXPRESSION.

Maryam Rezai Rad, Shireen A. Hafez, shaomian Yao
Louisiana state university, Department of Comparative biomedical sciences, Baton Rouge, LA, United States

Background and objectives: Although glutamine is considered as a non-essential amino acid, it may become essential under certain conditions, such as injury or illness. The medicinal benefits of glutamine have been recognized in the treatment of injury and in postoperative recovery. In addition, glutamine has been found to promote the proliferation of adult stem cells (ASCs). Together with the fact that ASCs contribute to the process of wound healing, it is our objective to determine if glutamine plays roles in differentiation of ASCs; particularly if glutamine can promote osteogenesis of dental follicle stem cells.
Methods: Dental follicle stem cells (DFSCs) were established from the dental follicle of first mandibular molar of postnatal rats. DFSCs at passages 3-5 were incubated in osteogenic medium containing 0, 1 and 2 mM L-glutamine for induction of osteogenesis. After 7 or 14 days of induction, cells were stained with Alizarin Red solution. Cells were also collected to determine the expression of osteo-induction genes using real-time RT-PCR. In a separate experiment, bone morphogenetic protein 2 (BMP2) and bone morphogenetic protein 6 (BMP6) were added to glutamine-free osteogenic medium to test their effect on induction of osteogenesis of DFSCs.
Results: Osteogenesis was occasionally seen after 7 days of induction in medium containing 2 mM glutamine, but not in medium containing 0 or 1mM glutamine. However, osteogenesis was observed after 14 days of induction in all treatments, in which increased osteogenesis was coincident with the increase of glutamine concentration. Real-time RT-PCR determined that glutamine significantly increased expression of BMP2 and BMP6 in the DFSCs during osteogenesis induction. When DFSCs were incubated in glutamine-free osteogenic medium containing BMP2 and/or BMP6, increased osteogenesis was observed, and the maximal osteogenesis occurred when both BMP2 and BMP6 were present.
Conclusion: This study indicates that glutamine can enhance the osteogenic capability of DFSCs. This effect is most likely due to up-regulation of the expression of BMP2 and BMP6.
Significance: The results are significant for the therapeutic use of DFSCs, as well as other ASCs. In particular, supplementation of glutamine would be helpful to achieve optimal therapeutic effects in stem cell therapy. The findings of this study can help in understanding the beneficial effect of glutamine in treatment of injury and in postoperative recovery.

T-2025

MURINE LONG-TERM MULTI-LINEAGE RENEWAL MARROW STEM CELLS ARE ACTIVELY CYCLING.

Laura Goldberg[1], Mark Dooner[1], David Adler[1], Elaine Papa[1], Michael Del Tatto[1], Mandy Pereira[1], Jason Aliotta[2], Peter Quesenberry[1]
[A]Medicine, [1]Rhode Island Hospital, Providence, RI, United States

The cell cycle status of hematopoietic stem cells (HSCs) has long been the subject of intense study. Based on an impressive body of work, conventional wisdom now holds that HSCs are predominantly quiescent in nature. However, almost all studies to date examining the cell cycle status of HSCs have focused solely on highly purified populations of stem cells. Here, we report studies exploring the cell cycle status of the total population of HSCs in unseparated whole bone marrow (WBM). Using Hoechst 33342 and Pyronin Y staining, we separated WBM into cell cycle-specific fractions using fluorescence activated cell sorting. We competitively engrafted each fraction into lethally irradiated host mice and determined donor contribution to chimerism by flow cytometry up to one year post-transplant. In these experiments, there was a clear population of cells in S/G2/M capable of long-term multilineage engraftment. There was no statistically significant difference in the percent donor chimerism between cells that were in G0/G1 compared to cells that were in S/G2/M at the time of transplant. In addition, the cycling stem cells in WBM retained their stem cell potential in secondary transplant. This is in stark contrast to published studies showing that highly purified stem cell populations such as LT-HSC (Lineage$^-$c-kit$^+$sca-1$^+$flk2$^-$) engraft predominantly when in G0. The presence of actively cycling HSCs was verified using tritiated thymidine (^{3}H-thymidine) suicide. WBM incubated with ^{3}H-thymidine or unlabeled thymidine was competitively engrafted into lethally irradiated mice and percent donor contribution to engraftment was determined by flow cytometry. In four separate experiments, there was a 65-80% reduction in engraftment by WBM incubated in the presence ^{3}H-thymidine. As ^{3}H-thymidine selectively kills cells going through S-phase, this reduction supports the specific loss of an actively cycling population HSCs capable of long-term engraftment. Given that highly purified stem cells engraft predominantly when quiescent but, as reported here, WBM contains actively cycling stem cells, it follows that the population of cycling marrow stem cells within WBM are lost with purification. We hypothesize that conventional stem cell isolation procedures relying heavily on stable cell surface epitope expression might skew selection toward more dormant populations with stable surface marker profiles. In contrast, the continually changing phenotype of cycling HSCs with cell cycle transit would render this population difficult to purify. In support of this, our preliminary data revealed that incubation of the Lineage-positive and Lineage-negative cellular fractions with ^{3}H-thymidine led to dramatic reductions in long-term multi-lineage engraftment potential within both populations (over 95% and 85% reduction, respectively). This indicates that the discarded population of stem cells during antibody-based stem cell purification is composed largely of cycling cells. In sum, these data support that 1) whole bone marrow contains actively cycling stem cells capable of long-term multi-lineage engraftment, and 2) the protean phenotype of actively cycling cells as they transit through cell cycle may render cycling marrow stem cells difficult to purify with conventional antibody-based methods. These data underscore the need to re-evaluate the total HSC pool on a population level in addition to a clonal level in order to provide a more comprehensive study of HSC biology.

T-2026

TARGETED NATURAL KILLER CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS AS A NOVEL STRATEGY TO TREAT HIV/AIDS

Zhenya Ni, David A, Knorr, Laura Bendzick, Dan S. Kaufman
Department of Medicine, Stem Cell Institute, University of Minnesota, Minneapolis, MN, United States

Stem cell-based therapies have been gaining significant interest for their ability to treat and potentially cure HIV/AIDS. Here, we have evaluated the potential to utilize human pluripotent stem cells, both human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), to derive lymphocytes that can be targeted to more effectively kill HIV-infected cells. hESCs and iPSCs can routinely differentiate into most all blood linages, including NK cells and T cells. Our previous studies have shown hESC and iPSC-derived NK cells can kill HIV-infected targets in vitro. Here, we advance those studies by expression a CD4ζ fusion protein in hESC/iPSC-derived NK cells. The CD4 receptor on hematopoietic cells is known to interact with HIV coat protein gp120. Earlier studies have utilized a

fusion protein combining CD4 extracellular protein to the CD3ζ intracellular signaling chain as a means to target and activate lymphocytes to potentially kill HIV infected cells. We hypothesized that expression of this CD4ζ fusion protein would more efficiently direct hESC and iPSC-derived NK cells to target and kill HIV infected cells. In these studies, we transduced CD4ζ into undifferentiated hESCs and iPSCs. Subsequent studies demonstrated stable CD4ζ expression and function in NK cells derived from these gene modified human pluripotent stem cells. Initial in vitro studies using T cell lines and primary CD4 T cells infected with HIV as targets suggested that both CD4ζ expressing hESC- and iPSC-NK cells inhibited HIV replication more efficiently than their unmodified NK cell counterparts. We then evaluated CD4ζ-hESC- and iPSC-NK cells for in vivo anti-HIV activity using a PBL-NSG mouse model. We demonstrated significantly greater suppression of HIV replication in different organs of the mice treated with either CD4ζ-modified or unmodified NK cells derived from either hESCs or iPSCs, compared to mice without NK cell treatment. Notably, we did not observe a significant difference in anti-viral suppression between groups treated with CD4ζ-hESC/iPSC-NK cell and wild type hESC/iPSC-derived NK cells. These studies establish a novel system to understand and direct innate immunity against HIV in early infection. The results also indicate that a hESC and iPSC-based combination of immune and gene therapy can be utilized as a unique resource to target HIV/AIDS or other chronic viral infections.

Technologies for Stem Cell Research

T-2027

EXPANSION OF ADIPOSE DERIVED MESENCHYMAL STEM CELLS USING SHAKE FLASKS

Khandaker Siddiquee, Ma Sha
Eppendorf, Enfield, CT, United States

Mesenchymal stem cell (MSC) is a type of multipotent adult stem cell that can differentiate into many specialized cell types including adipocytes, skin cells, tendons and muscle cells, osteoblasts, and chondrocytes. MSC based therapy has significant clinical potential in many areas such as organ transplant rejection, spinal cord injury, liver injury, multiple sclerosis etc... Currently, there are over 300 clinical trials exploring the use of MSCs for the treatment of various diseases. The expansion of stem cells, including MSCs, has been successfully demonstrated using microcarrier based small bioreactors, such as spinner flasks. In this study, we explored a simple alternative for the microcarrier based MSC expansion using conventional shake flasks. The method relies on a new type of shaker with built-in CO2 gas control capability (Incubator Shaker). The MSC expansion was compared between shake flasks and spinner flasks using microcarriers or Fibra-Cel® disks. The MSC cells were seeded at a density of $3X10^4$ cells/cm^2, into either a baffled shake flask or a spinner flask, each containing 0.5 g of plastic microcarriers (cross-linked polystyrene) and 40 ml of stem cell growth medium (serum and growth supplement free). Another shake flask was seeded similarly except with 1.0 g of Fibra-Cel® disks in place of microcarriers. For the initial attachment of cells, the agitation speed of the Incubator Shaker and rotation speed of the spinner (Housed inside of a CO2 incubator) were kept to 50 rpm with both CO2 control set to 5%. After 1 hour of incubation, 10 ml each of 10% serum, 25 ng/ml growth supplement and 12 mM L- glutamine were added to the culture to reach a final concentration of 2% serum, 5 ng/ml growth supplement and 2.4 mM L- glutamine. In the meanwhile, the agitation speed of both systems was raised to 75 rpm. The MSC culture expansion continued on for 5 days. Samples were collected daily from both spinner and shake flask cultures to monitor cell count, cell viability, glucose consumption and lactate production. The preliminary results indicate that MSC can be expanded successfully using conventional shake flasks with cell density equal or better than that of the small spinner bioreactors. Microcarrier based MSC culture expansion using shake flasks achieved higher cell density ($8.8X10^5$ cells/cm^2) as compared to the spinner flask ($3.6X10^5$ cells/cm^2) during the early log phase of the growth. Additional experiments are planned to assess the reproducibility and statistical significance. The Fibra-Cel® disks based MSC culture using shake flasks also achieved similar cell density as the microcarrier shake flasks.
MSC expansion using shake flasks appears to be a viable and simple alternative to the spinner flask system. This method reduces experimental complexity and decreases the risk of contamination associated with inserting non-

sterile magnetic stirrer base into a CO2 incubator, a common practice for spinner flask based mammalian cell culture.

T-2028

AUTOMATION OF CELL THERAPY BIOMANUFACTURING: MINIMIZING REGULATORY RISKS AND MAXIMIZING RETURN ON INVESTMENT

David A. Brindley[1], Ivan Wall[3], Richard W. Barker[2], Brock Reeve[4], Kim Bure[5], Andrew J. Carr[1]

[A]Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, [B]Centre for the Acceleration of Sustainable Medical Innovation, [1]Oxford University, Oxford, United Kingdom, [2]Department of Biochemical Engineering, University College London, London, United Kingdom, [3]Harvard Stem Cell Institute, Harvard University, Cambridge, MA, United States, [4]TAP Biosystems, Royston, United Kingdom

ISSCR Conference Abstract: *Automation of Cell Therapy Biomanufacturing: Minimizing Regulatory Risks and Maximizing Return on Investment*

Track: Stem Cells and Tissue Engineering and Technologies for Stem Cell Research

Study Objective: The objective of the study was to quantitatively and qualitatively evaluate the regulatory and financial implications of automating cell therapy biomanufacturing processes.

Brief Statement of Methods: Firstly, a review of existing literature and targeted interviews with academic and industry leaders was performed to identify the key scientific, bioprocessing and regulatory challenges associated with cell therapy biomanufacturing automation. Secondly, quantitative process simulation studies were conducted on an anonymised bioprocess for two cell types at two different manufacturing scales: an autologous MSC-like cell line and an autologous dentritic cell-like cell line, both at small scale (350 patients) and large scale (1000 patients).

Summary of Results Obtained: For both cell types there was in excess of a tenfold decrease in particulate levels when using a functionally closed robotic processing chamber, versus typical manual culture techniques. Further, an decrease in cell population variability enabled a number of number of cell based assays, including a radioligand binding assay, to produce statistically significant results which was not possible when utilizing cells produced from manual culture cell techniques. At all scales, and all cell types, there was a reduction in the cost per dose when automated biomanufacturing techniques were employed. This trend was most notable for large scale autologous immunotherapy manufacture, where potential cost savings of approximately $2.85m was demonstrated – with a capital investment payback period of less than nine months.

Statement of Conclusions: It is evident that well designed and implemented automated cell biomanufacturing techniques can reduce cell population heterogeneity and produce valuable data to support regulatory submissions as products progress from lab-bench to the clinic. However, it is challenging to design bioprocessing technologies that adequately accommodate the inherent physiological sensitivity of human cell lines due largely to intrinsic telomere dynamics, difference in donor ages, oxidative stress status, and stochastic events.

T-2031

GENOME WIDE COMPARATIVE ANALYSES OF RETROVIRAL AND DNA TYPE TRANSPOSON VECTOR INTEGRATION SITES IN MOUSE EMBRYONIC STEM CELLS: IMPLICATION FOR REPROGRAMMING STUDY

Junko Yoshida[1], Kyoji Horie[2]

[1]Graduate Sch of Medicine, Osaka Univ., Suita, Japan, [2]Physiology II, Nara Medical Univ., Kashihara, Japan

Gene transfer is an essential and indispensable technique for reprogramming study. Although integration-free vectors are preferable for safety reasons, stable vector integration is inevitable in many situations, e.g. direct reprogramming into specific cell types. Various vectors have been used for reprogramming, such as retrovirus, piggyBac (PB) transposon, Tol2 transposon, and Sleeping Beauty (SB) transposon. To achieve reprogramming in an efficient and safe manner, we need to know characteristic of each vector, such as gene transduction efficiency and insertion site preference.

In the present study, we conducted large-scale comparative analyses of the above-mentioned four vectors in mouse embryonic stem cells using high-throughput DNA sequencing technologies. We tried to minimize any bias during determination of insertion site sequences, and analyzed them in various respects, such as hotspot insertion site, relative distance to RefSeq genes or transcription start site (TSS), histone modifications, gene expression, GC contents, RpolII binding sites and gene ontology.
Bioinformatic analysis revealed substantial differences between four vectors. PB has strong preference to actively transcribed regions, although not as pronounced as retrovirus. Only SB does not have preference to TSS. Tol2 insertion sites are correlated with a specific histone modification. There are many other differences that will be presented in the meeting. Our results provide a framework for differential utilization of retroviral and transposon vectors depending on the purpose of the study.

T-2032

DEVELOPMENT OF FRET BASED PROBE TO DETECT CONFORMATIONAL CHANGE OF INTEGRIN α4β1 FOR VISUALIZING CELL ADHESION

Yuka Sugihara, Yuriko Higuchi, Yoshimasa Kozu, Shigeru Kawakami, Fumiyoshi Yamashita, Mitsuru Hashida
Department of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

Cell adhesion and migration are essential process of inflammation, tumor growth, and tissue repair. The purpose of this study is to visualize the cell adhesion. Very late antigen-4 (VLA-4, integrin α4β1) expressed on mesenchymal stem cells regulates homing and tissue selective adhesion to endothelial cells (ECs). In these processes, integrin conformational changes between bent and extended conformation decide binding affinity to its counter structure on ECs. Cytoplasmic domains of integrin α and β become close to each other in a bent conformation during integrin-inactive state. On the other hand, cytoplasmic domains of integrins are separate in the extended conformation when integrins are activated by ligand binding. Therefore, the state of VLA-4 would be detected by visualizing the relative distance between the cytoplasmic domains with fluorescence resonance energy transfer (FRET). In this study, we developed FRET-based probe for monitoring cell adhesion.
Plasmid DNA expressing of fusion proteins, such as integrin α4 and mCFP (α4-mCFP) or integrin β1 and mYFP (β1-mYFP), were established by the insertion of mouse integrin α4 and β1 cDNA into the ends of the N-terminus of the monomeric variant CFP and YFP, respectively. For α4-mCFP, integrin α4 and mCFP were conjugated with the linker of (GGGGS)3 based on the previous report (Methods Cell Biol. 85, 381, 2008). For β1-mYFP, integrin β1 and mYFP were conjugated with the linker of (GPVAT)3 based on the previous report (Biochemistry 37, 9918, 1998).
After transfection of either plasmid DNA into HeLa cells using FuGENE 6, expressions of α4-mCFP or β1-mYFP in cells were confirmed by western blotting methods using polyclonal anti-GFP antibody. Cells transfected either or both plasmid DNA were observed by fluorescence microscopy, and then FRET efficiency was evaluated by photobleaching method. At first, we optimized the linker sequence. As for α4-mCFP, fluorescence intensity of α4-(GGGGS)3-mCFP was much higher than that of α4-(SGGGG)3-mCFP; therefore we used the former fusion protein in the following study. As for β1-mYFP, fluorescence intensity of β1- (GPVAT)3-mYFP was bright enough. We also confirmed that the both expression of α4-mCFP and β1-mYFP were localized in cytoplasm and plasma membrane. These distributions of fluorescence signal were similar with the distribution of endogenous integrin α4 of myeloid cells or integrin β1 of HeLa cells. Dequenching of YFP (ex. 438 nm) was observed after CFP was photobleached. And, dequenching of YFP and a slight recovery of CFP (ex. 438nm) were observed after YFP was photobleached. Theses result suggested that FRET was observed between α4-mCFP and β1-mYFP although FRET efficiency was still extremely week. Optimization of donor/acceptor combination should improve FRET efficiency of this FRET-based probe.

T-2033

EVALUATING THE LANDSCAPE OF SPECIFICITY OF CRISPR-CAS SYSTEMS FOR RNA-GUIDED HUMAN GENOME ENGINEERING

Prashant Mali, John Aach, George M. Church

Harvard Medical School, Boston, MA, United States

Bacteria and archaea have evolved adaptive immune defenses termed clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems that use short RNA to direct degradation of foreign nucleic acids. Recently we demonstrated that the type II bacterial CRISPR system can be engineered to function with custom guide RNA (gRNA) in human cells, effecting robust genomic editing across multiple targets and in multiple cell types, including human induced pluripotent stem cells (iPSCs). Specifically, for the endogenous AAVS1 locus we obtained targeting rates of 10-25% in 293Ts, 13-38% in K562s, and 2-4% in iPS cells, showing successful homology direct repair using both conventional double-stranded DNA donors and short 90mer oligonucleotides. Importantly, upon simultaneous introduction of multiple gRNAs we could successfully effect efficient multiplex editing of target loci. While the ease of retargeting this system to modify genomic sequences greatly exceeds that of comparable zinc-finger nucleases (ZFNs) and TAL effector nucleases (TALENs), elucidating the frequency and underlying causes of off-target nuclease activity induced by CRISPR, ZF, and TALE genome-engineering tools is of utmost importance for targeted genome modification, and ultimately safe gene therapy. Towards this we develop here a novel next-generation sequencing (NGS) based assay to compute a comprehensive landscape of candidate off-targets, comparing both CRISPR efficacy and corresponding TALEs targeting similar loci. Our approach employs a biased library of CRISPR and TALE targets transfected into cells. Their relative targeting is assayed by NGS, and the off-targeting landscape is thence evaluated. For this analysis of specificity we explored multiple gRNAs with varying distributions of nucleotide content, and also evaluated TALEs of multiple lengths (18-mer, 14-mer and 10-mer proteins). Our results provide insights into the rules of type II CRISPR specificity: in particular the role of the protospacer-adjacent motif (PAM), the 8- to 12-base “seed sequence” at the 3’ end of the gRNA, and the importance of the remaining 8 to 12 bases on the binding strength of the matching gRNAs. Correspondingly, evaluations of TALE specificity provide insights into their tolerance to mutations and importantly also the impact of their relative positions in the targeting sequences. We are currently evaluating strategies to improve specific gene-targeting using both these programmable systems. Overall, our results demonstrate the promise of CRISPR-mediated RNA-guided targeting for facile, robust, and multiplexable human genome engineering. We believe this tool will have broad implications for the direct and multiplexed perturbation of gene networks, and for targeted ex vivo and in vivo gene therapy applications.

T-2034

SUCCESSFUL CULTURE OF ADHERENT CELL TYPES IN A FUNCTIONALLY CLOSED AUTOMATED CELL EXPANSION SYSTEM USING MULTIPLE FREEZE-THAWED CRYOPRECIPITATE AS A BIOREACTOR MEMBRANE COATING REAGENT

Michelle M. Brecheisen, Brian J. Nankervis, Kim Nguyen
Terumo BCT, Lakewood, CO, United States

The Quantum Cell Expansion System (Quantum System) is a functionally closed and automated hollow-fiber bioreactor system that is designed to reproducibly expand adherent cells in either Good Manufacturing Practice (GMP) or research laboratory environments. The hollow-fiber membrane requires a coating reagent to help facilitate cellular adherence when culturing an adherent cell type, such as mesenchymal stem cells (MSC). Pooled human cryoprecipitate (CPPT, Bonfils Blood Center) was examined as an alternative to Fibronectin (FN, BD Biosciences) because it is a rich source of extracellular matrix components, including fibrinogen (Freedman, 2010), and also because it was previously shown to help aid cellular adherence in tissue culture flasks (Nikolaychik, 1994). In a previous study, minimal variation between clinical scale harvests of pre-cultured MSC was observed between volumes as low as 1/100 of a constant single donor equivalent (SDE) volume of CPPT (Peters, 2012). Up to 475 mL of CPPT diluted to a constant SDE volume is discarded after only one freeze-thaw event at -20°C. To utilize CPPT in a more cost efficient manner, it has now been demonstrated that CPPT exposed to a second freeze-thaw event can still be used as a bioreactor membrane coating reagent for *ex vivo* expansion of adherent cell types on the Quantum System.
Three different adherent cell types, adult bone marrow-derived mesenchymal stem cells (BM-MSC), adult normal human dermal fibroblast (NHDF), and adult human adipose-derived mesenchymal stromal cells (ASC) were expanded on the Quantum System using CPPT as the bioreactor membrane coating reagent that was exposed to -20°C for one freeze-thaw event and two freeze-thaw events. In both conditions, 25 mL of CPPT that was diluted to a

constant SDE volume in Phosphate Buffered Saline (PBS) was further diluted to 100 mL of PBS and then used to coat the bioreactor. The standard Terumo BCT protocols developed for the culture of BM-MSC, NHDF, and ASC were utilized and consisted of automated tasks such as bioreactor coating, cell loading, attachment, feeding, and harvest. The Quantum System expanded MSC met all typical MSC characteristics according to the position paper from the International Society for Cellular Therapy (ISCT) (Dominici, 2006) in terms of morphology, phenotype, and differentiation. In addition, assays were developed to characterize the condition of CPPT after exposure to multiple freeze-thaw events and resulted in minimal impact on CPPT condition. Experimental results demonstrate that robust *ex vivo* expansion of BM-MSC, NHDF, and ASC is feasible using multiple freeze-thawed CPPT as a bioreactor membrane coating reagent on the Quantum System to reach clinically relevant cell yields.

T-2035

ESTOOLS DATA@HAND, A GENE EXPRESSION DATA ANALYSIS RESOURCE FOR STEM CELL RESEARCH

Kaisa-Leena Aho[1], Lingjia Kong[1], Kirsi Granberg[1], Riikka Lund[2], Laura Järvenpää[1], Janne Seppälä[1], Paul Gokhale[3], Elisa Närvä[2], Peter W. Andrews[3], Matti Nykter[1], Riitta Lahesmaa[2], Christophe Roos[1], Reija Autio[1]
[1]Department of Signal Processing, Tampere University of Technology, Tampere, Finland, [2]Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, Turku, Finland, [3]Centre for Stem Cell Biology and the Department of Biomedical Science, University of Sheffield, Sheffield, United Kingdom

Gene expression array data, extensively produced within stem cell research, can be exploited to resolve new questions when analysed from different perspectives. However, means to perform large-scale analyses across data published by different studies are limited. We have developed ESTOOLS Data@Hand, a publicly available computational web resource that provides an exceptionally large set of expression array data and efficient analysis tools for the studies of stem cell pluripotency, differentiation and cell de-differentiation. The ESTOOLS Data@Hand resource regroups published gene expression data from Gene Expression Omnibus and ArrayExpress and contains more than 1200 samples from 77 sample sets. They cover different pluripotent cells as well as dozens of other cell and tissue types reported in the same studies in various conditions. The power of the resource is increased by structured, manually curated annotations for each sample gathered from publications and authors. These include 60 annotation dimensions concerning the characteristics of the cells, treatments, used array protocols and literature citations. All annotations can be used to efficiently query samples in the resource, to select data for analysis, and to order samples in visualisations. The data is pre-processed, and all measures are linked to Ensembl and HGNC gene name space to enable comparison of results from different sample sets. A total of 408 Affymetrix and 245 Illumina array samples have been pre-processed jointly into two sample meta-sets. These meta-sets enable cross-experiment analyses. Data analysis tasks are easy to perform as workflows. The workflows enable analysis of gene expression data using state of the art methods: finding co-expressed genes across selected samples, differentially expressed genes between sample groups, enrichment analysis of GO terms and KEGG pathways, and clustering. Visualisations include heatmaps and expression profiles. In summary, ESTOOLS Data@Hand is a powerful one-stop site for stem cell transcriptomics.

T-2036

IMPROVED METHODS FOR REPROGRAMMING HUMAN DERMAL FIBROBLASTS USING FLUORESCENCE ACTIVATED CELL SORTING

David J. Kahler, Faizzan S. Ahmad, Anita Ritz, Haiqing Hua, Dorota N. Moroziewicz, Andrew A. Sproul, Carmen R. Dusenberry, Linshan Shang, Daniel Paull, Matthew Zimmer, Keren A. Weiss, Dieter Egli, Scott A. Noggle
The New York Stem Cell Foundation, New York, NY, United States

Current methods to derive induced pluripotent stem cell (iPSC) lines from human dermal fibroblasts by viral infection rely on complex protocols. One major factor contributing to the time required to derive lines is the ability of researchers to identify fully reprogrammed unique candidate clones from a mixed cell population containing

transformed or partially reprogrammed cells and fibroblasts at an early time point post infection. Failure to select high quality colonies early in the derivation process results in cell lines that require increased maintenance and unreliable experimental outcomes. Here, we describe an improved method for the derivation of iPSC lines using fluorescence activated cell sorting (FACS) to isolate single cells expressing the combination of ***CD13^{NEG}SSEA4^{POS}Tra-1-60^{POS}*** cell surface markers between 7-10 days post infection. This technique prospectively isolates fully reprogrammed iPSCs, and depletes both parental and "contaminating" partially reprogrammed fibroblasts, substantially reducing the time and reagents required to generate iPSC lines. iPSC lines derived under this technology produced more unique and stable clones following retroviral infection than manual picking methods, expressed common markers of pluripotency at later passages, and possessed spontaneous differentiation potential *in vitro* and *in vivo*. FACS derivation produced iPSC lines had a normal karyotype and matched the parental DNA fingerprint. To demonstrate the suitability of FACS for high-throughput iPSC generation, we derived 228 individual iPSC lines from a variety of 76 tissue sources using either integrating (retroviral) or non-integrating (Sendai virus) reprogramming vectors and performed extensive characterization on a subset of those lines.

T-2037

KINETICS AND EPIGENETICS OF RETROVIRAL SILENCING IN MOUSE EMBRYONIC STEM CELLS DEFINED BY DELETION OF A D4Z4 BARRIER ELEMENT

Sylvie Rival-Gervier[1], Mandy Y.M. Lo[1], Shahryar Khattak[1], Peter Pasceri[1], Matthew C. Lorincz[2], James Ellis[1]
[1]Developmental and stem cell biology, Hospital For Sick Children, Toronto, ON, Canada, [2]Medical Genetics, University of British Columbia, Vancouver, BC, Canada

Retroviral vectors are silenced in pluripotent stem cells. This feature facilitates the characterization of fully reprogrammed induced pluripotent stem (iPS) cells. However, silencing of retroviral vector remains the major concern for many other applications such as gene transfer and cell therapy, recombinant protein production or human disease models using stem cells. Our goals are to maintain long term, predictable and stable expressions of transgene and to decipher transgene silencing mechanisms in pluripotent stem cells. The D4Z4 element is a repeated subtelomeric element involved in Facio-Scalpulo-Humeral Dystrophy and has been described as a novel insulator: It can protect transgenes from silencing; it blocks the interaction between enhancer and promoter when it is located between them. Its activity is dependent on CTCF and A-type lamin. We investigated whether D4Z4 is able to protect retrovirus vector transgenes from silencing in mouse embryonic stem cells and induced pluripotent stem cells.
We analyzed the expression from self-inactivated retroviral vectors bearing an EF1α-EGFP-ires-Puromycin reporter over 5 months in culture. We compared the basic unprotected vector to versions insulated by cHS4 insulator in the LTRs and/or D4Z4 subfragments clones between *gag* and the EF1α promoter. We show here that a 3'D4Z4 insulator directs retroviral expression with persistent but variable expression for up to 5 months. Combining an internal 3'D4Z4 with HS4 insulators in the LTRs shows that these elements cooperate, and defines the first retroviral vector that fully escapes long-term silencing. Using FLP recombinase to induce deletion of 3'D4Z4 from the provirus in ES cell clones, we established retroviral silencing at many but not all integration sites. This finding shows that 3'D4Z4 does not target retrovirus integration into favourable epigenomic domains but rather protects the transgene from silencing. Chromatin analyses demonstrate that 3'D4Z4 is a barrier element that blocks the spread of heterochromatin marks including DNA methylation and repressive histone modifications such as H3K9 methylation. In addition, our deletion system reveals three distinct kinetic classes of silencing (rapid, gradual or not silenced), in which multiple epigenetic pathways participate in silencing at different integration sites. We conclude that vectors with the 3'D4Z4 barrier and HS4 insulator elements block silencing, and may have unprecedented utility for gene transfer applications that require long-term gene expression in pluripotent stem cells. Moreover, our use of FLP to delete 3'D4Z4 and then to induce silencing is a definitive method to identify barrier elements, to investigate the kinetics of epigenetic events during retrovirus silencing and provides a tool for screening chemicals that can inhibit these pathways.

T-2038

TALEN MEDIATED GENOME ENGINEERING OF HUMAN PLURIPOTENT STEM CELLS

William T. Hendriks[1], Laurence Daheron[2], Kiran Musunuru[1], Chad A. Cowan[1]

[1]Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States, [2]Harvard Stem Cell Institute, Cambridge, MA, United States

Using Transcription Activation Like Effector Nuclease (TALEN)-mediated genome editing, we can create human pluripotent stem cell (hPSC) lines with knockout or mutant alleles that can be used for differentiation into various cell types. This strategy of genome engineering in hPSCs will prove invaluable for studying human biology and disease. TALENs are arrays of 34-amino-acid DNA binding domains fused to FokI-nuclease domains. These arrays can be easily engineered to bind almost any DNA sequence. Introduction of a pair of TALENs into hPSCs creates a double-strand break (DSB) in the targeted gene that is either repaired by error-prone non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ typically results in frameshift mutations in the targeted locus, generating a knockout hPSC line when both alleles are targeted. Simultaneous delivery of a TALEN pair and a DNA donor template can result in HDR and can be used to introduce specific mutations, repair endogenous mutations in disease-specific hPSC lines, or create hPSC reporter lines. Using TALEN technology, we targeted LINC116 (non-coding RNA) and TTN (titin), postulated to play roles in adipogenesis and cardiomyopathy, respectively. The targeting efficiencies for LINC116 and TTN were 29.5% and 6.8%, respectively. Only one round of targeting was sufficient to generate knockout lines for both genes. Currently, we are targeting MECP2 (methyl CpG binding protein 2) with a single stranded oligodeoxynucleotide (ssODN) in order to introduce a R306C mutation, implicated in Rett Syndrome. Our pilot studies reveal that this novel strategy is a far more efficient tool for genome engineering in hPSCs compared to conventional gene targeting strategies. Furthermore, the modular nature of TALEN provides more flexibility in the design of DNA-binding proteins compared to Zinc Finger Nucleases or Meganucleases.
TALEN-mediated genome engineering in hPSCs is available as a fee-for-service offered through the Harvard Stem Cell Institute iPS Core Facility.
This study was funded by the Qatar National Research Fund (NPRP 09-1147-3-284-3-284)

T-2041

GRAVITY ENVIRONMENT CHANGING IS A KEY FACTOR FOR MUSCLE DIFFERENTIATION REGULATIO

Yumi Kawahara[1], Kumi Morikawa[2], Masaya Matsumoto[2], Takahiro Fukazawa[2], Takeshi Imura[2], Elham Khalesi[2], Ya-Nan Sun[2], Hiroyuki Uwatoko[2], Kyousuke Nakata[2], Yunosuke Ookura[3], Takuma Furukawa[3], Louis Yuge[2]

[1]Space Bio-Laboratories Co. Ltd., Hiroshima, Japan, [2]Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan, [3]Institute of Health Sciences, Hiroshima University, Hiroshima, Japan

Gravity is a physical stimulus with strong influence in the development of life. It has been found that the cell differentiation of myoblasts is suppressed during space flights and weightlessness. The 3D-clinostat, a simulated microgravity machine, is a multi-directional gravity device controlled by rotation of two axes, and makes 10^{-3} G average over time. Here we demonstriated that the effects of gravity on muscle differentiation, when myoblasts exposed to microgravity and returned them to gravity during differentiation. L6 rat myoblast cells propagated in normal 1G environment. When L6 cells reached confluence on culture day 4, they were induced differentiation and then exposed to microgravity environment (group CL). Control cells were cultured in normal 1G environment (group 1G). After 5 days induced differentiation, cells were divided into two more groups and cultured for another 5 days. Subject was continued to culture in same environment (group CL/CL or group 1G/1G), the other subject was exchanged culture environment of the cells (group CL/1G or group 1G/CL), thus cells in group CL were transferred to 1G, and cells in group 1G were transferred to microgravity. Morphological observation and molecular biological analysis indicated that microgravity inhibited myoblast differentiation. The cells in group CL/1G accelerated differentiation when cells were returned to normal 1G environment, while the cells in group 1G/CL suppressed differentiation, when cells were put in microgravity environment. Interestingly, in group 1G/CL, differentiated myotubes seemed to turn back to myoblasts, what is called dedifferentiation. Moreover, these gravitational biological changes influenced $p38^{MAPK}$

cascade activated or inactivated transduction pathway to control myoblast differentiation. Gravity really regulated myoblast differentiation. The muscle differentiation regulation using gravity is a novel approach for muscle regeneration.

T-2042

ANDROGENETIC HAPLOID EMBRYONIC STEM CELLS SERVE AS NEW TOOLS FOR GENETIC MODIFICATIONS IN CELLULAR AND ANIMAL LEVEL

Wei Li, Ling Shuai, Xin Li, Haifeng Wan, Mingzhu Dong, Tianda Li, Minggui Jiang, Meng Wang, Guan-Zheng Luo, Liu Wang, Xiu-Jie Wang, Xiao-Yang Zhao, **QI ZHOU**
INSTITUTE OF ZOOLOGY, CHINESE ACADEMY OF SCIENCES, BEIJING, China

Haploid cells which lack of allelic genes have great implication in studying recessive traits. However, natural haploids are rare in animals, usually exist in germ cells and are occasionally found in tumors with massive chromosome loss. Recently medaka fish and mouse haploid embryonic stem (ES) cells have been successfully established, raised the possibility of using engineered mammalian haploid cells in genetic studies. However, the developmental ability and genetic function of mammalian haploid ES cells have not been fully characterized, including whether the haploid nature of ES cells can be steadily maintained in further-differentiated cells, whether the haploid ES cells can function as haploid gametes to support fertilization and development, and whether genetic modifications in the haploid ES cells can be transmitted properly to offspring. To address these questions, we established androgenetic haploid ES (ahES) cell lines by transferring sperm into an enucleated oocyte. The haploid ES cells can maintain haploidy with only one intact chromosome set for more than 30 passages, express pluripotent marker genes, possess the ability to differentiate into all three germ layers, and contribute to the germlines of chimera when injected into the blastocysts. Although epigenetically distinct from sperm cells, the ahES cells can produce viable and fertile progenies after intracytoplasmic injection into mature oocytes. These characteristics make ahES cells an easy-to-manipulate system for genetic modifications. High-throughput genome wide mutations by the piggyBac transposon and precise gene targeting via homologous recombination are obtained in ahES cells. Moreover, the oocyte-injection procedure can also produce viable transgenic mice from genetically engineered ahES cells, which have normal fertility to further transfer the transgenes to next generations. Of note, the ahES cells could differentiate under the haploid state both *in vivo* and *in vitro* initially after differentiation, but rapidly go diploidized in further differentiation. Our findings clarify the developmental characteristics of ahES cells, and demonstrate their application as new tools for genetic modifications in both cellular and animal level. These studies also shed new light on genetic screening and transgenic model production via ahES cells in other species including non-human primates, as well as assisted reproduction.

T-2043

A STRATEGY FOR EMBRYO-TO-ADULT AGM TRANSPLANTATION IN ZEBRAFISH

Gregory Frechette, Virginie Esain, Trista North, Wolfram Goessling
Pathology, Beth Israel Deaconess Medical Center, Boston, MA, United States

In the mouse, long-term repopulating hematopoietic stem cells (HSC's) capable of sustaining multilineage engraftment of an lethally irradiated adult recipient are first derived from the hemogenic endothelium in the ventral wall of the dorsal aorta, within the Aorta-Gonad-Mesonephros region (AGM) between e10.5 and 11.5 of development. The expression of functional markers of HSCs, such as the transcription factor Runx1 which is required for definitive HSC formation, are highly conserved amongst vertebrate species. This conservation has been successfully used to identify novel regulators of vertebrate HSC development and function using a chemical genetic screening approach in zebrafish, resulting in the identification of the first compound isolated using zebrafish to be used in an FDA-approved clinical trial. While these results have been encouraging, it has not been formally shown that cells in the zebrafish AGM expressing HSC markers such as *runx1, cmyb* or *CD41* can act as functional HSCs *in vivo*. Using a chemical biology based approach, we have recently shown that modulations of physiological levels of glucose

produce dose-dependent effects on HSC development in the AGM region. In order to show that an apparent increase in HSC specific gene expression correlated to an increase in functional HSC's, we developed a novel zebrafish embryo-to-adult transplant model based on the original murine AGM transplantation experiments performed by Muller *et al,* (Immunity 1994). Prior to transplantation, we isolated the trunk region from CD41:eGFP+ transgenic reporter embryos, which maintain expression in the adult, by microdissection and disaggregated the cells manually after treatment with dithiothreitol (DTT). Five embryo equivalents of labeled wild-type (WT) or glucose-treated "donor" cells were injected intracardially along with 200,000 WT peripheral blood (PB) cells into lethally irradiated (25Gy) WT adult recipients. Fluorescent microscopy performed 3 weeks post transplant revealed a significantly increased number of recipients of glucose treated AGM cells displayed GFP+ cells present in the thymus, kidney marrow (KM) and spleen compared to recipients of control AGM cells. FACS analysis confirmed this finding showing a two-fold increase in GFP+ cells in the KM or PB of recipients of glucose treated embryonic AGM cells. Independent replicates showed GFP+ cells present at greater than 10 weeks post-injection indicating sustained repopulation in this assay; furthermore, multilineage engraftment potential was demonstrated in transplant recipients as marked by FCS/SSC FACS analysis of donor-derived CD45:dsRed+ cells in CD41:GFP+ expressing recipients. While further transplant studies are needed to reveal the precise embryonic cell types contributing to sustained hematopoietic engraftment, this novel embryo-to-adult transplant model shows great promise for examining the functionality of chemical or genetically-modified zebrafish AGM HSCs.

T-2044

TOOLS TO IMAGE AND COMPUTE THE HETEROGENEITY AND DYNAMICS OF OCT 4 EXPRESSION PATTERNS IN HUMAN PLURIPOTENT STEM CELL CULTURES

Kiran Bhadriraju[1], Joe Chalfoun[2], Michael Halter[3], Peter Bajcsy[2], Kye-Yoon Park[4], Barbara Mallon[4], John Elliott[3], Mary Brady[2], Anne Plant[3]

[1]Fischell Department of Bioengineering, University of Maryland, College Park, MD, United States, [A]Information Technology Laboratory, [B]Biosystems and Biomaterials Division, [2]National Institute of Standards and Technology, Gaithersburg, MD, United States, [3]Stem Cell Unit, National Institutes of Health, Bethesda, MD, United States

Pluripotent stem cell cultures exhibit heterogeneity in morphology and gene expression, which is difficult to quantify in a robust fashion due to the large colony size, and the small colony number, relative to the number of cells. This heterogeneity may provide insight into the fundamental biology of pluripotent stem cells, and it is essential for defining the metrics for assessing stem cell culture quality for therapeutic purposes. Imaging and associated computational tools can make such determinations possible. It was the objective of this study to build an experimental system, and a data visualization and analysis pipeline, which allow one to capture images of hundreds of stem cell colonies by automated fluorescent microscopy, visualize the image data, and track changes in the state of the cultures over several days. The H9 human embryonic stem cell line was engineered to produce GFP under the influence of Oct4 promoter and cells were cultured under feeder-free conditions on MatrigelTM. The culture dish was imaged in a mosaic of 252 contiguous frames covering approximately 180 square millimeters, over five days under both phase contrast and green fluorescence channels, with images acquired every 15 minutes. The large size of the image dataset required designing algorithms that can be executed on limited shared memory computers or on distributed computational resources. We have designed and implemented such algorithms for flat field correction, image stitching, Deep-Zoom pyramid building, colony segmentation, colony feature extraction, and colony tracking in order to enable web-based multi-scale visualization to display on-the-fly image data at different spatial scales, and to quantify colony and sub-colony morphology and dynamics. By tracking individual colonies over time, we were able to measure the time-course of colony growth, and shape fluctuations of individual colonies, as well as changes in the distribution of Oct4 promoter driven GFP expression in the cultures. Together, these set of tools provide an experimental and computational framework for following spatio-temporal colony characteristics as well as for the detection of colony events occurring at very low rates.

T-2045

OPTIMIZED EXPANSION AND CHARACTERIZATION OF HUMAN INDUCED PLURIPOTENT AND EMBRYONIC STEM CELLS WITHOUT COMPROMISING PLURIPOTENCY.

Amy D. Sinor-Anderson, Joanne Hasskamp, Crystal Zellefrow, Tina Marwood
Thermo Fisher Scientific, Rochester, NY, United States

The ability to generate sufficient relevant material for drug discovery efforts is critical in the study of certain diseases such as neurodegenerative disorders, where the traditional source material is typically primary tissue from animals. Such investigations require billions of cells and a viable alternative source for these studies are pluripotent stem cells. iPS and ES cells represent a valuable tool for both drug discovery and cell replacement therapies. One can use ES cells to generate large numbers of specific cell types needed to carry out drug discovery screening campaigns in order to understand the basic biology of diseased states. Currently, a major difficulty in the field is the lack of effective methods for growing, expanding, differentiating, and characterizing these cells. Optimization of cell cryopreservation, the cell media and growth surface, and characterization of the stem cell population could significantly accelerate drug discovery efforts. We have therefore developed effective methods for cryopreservation, growth, expansion, and characterization of ES and iPS cells. With this optimized approach, we can expand ES and iPS cells by 2 orders of magnitude over standard culture conditions. This ability to increase cell number in fewer passages will quicken the pace of drug discovery efforts. We have validated our protocols by comparing total cell counts, the number of cells that express Oct4 and Sox2 proteins, and the percentage of cells that express Oct4 and Sox2 to ensure their undifferentiated states. To determine the pluripotency of the stem cells expanded in our system, we differentiated these cells into the three germ layers: ectoderm, endoderm, and mesoderm. Finally, we confirmed karyotypic stability of the pluripotent stem cells after being cultured for 5-10 passages. Our methods reliably increase the expansion rate of pluripotent stem cells in fewer passages without compromising characteristic stem cell features.

T-2046

EX VIVO EXPANSION OF HUMAN AMNIOTIC FLUID STEM CELLS

Sophie S.-F. Hwan, Rebecca S. Gilbert, Jessie H.-T. Ni
R&D, Irvine Scientific, Santa Ana, CA, United States

Objective: Human amniotic fluid-derived stem cells (hAFSCs) could be a promising source of cell therapy application not only because of their multipotent stem cell characteristics and certain embryonic stem cell properties, but also due to their high capability of proliferation *in vitro*. The aim of this study was to optimize the culture medium for hAFSCs ex vivo expansion, and to characterize expanded cells based on the expression of stem cell markers and differentiation potency.
Methods: The isolated hAFSCs were cultured for over three passages in PRIME-XV™ hAFSC Expansion Medium, a complete serum containing medium, and then stained for flow cytometry and immunocytochemistry analysis. The expanded cells were also cultured in varied differentiation conditions for testing specific cell lineages.
Results: We found that hAFSCs could be expanded more than 100 fold over three passages in PRIME-XV™ hAFSC Expansion Medium. In addition, hAFSCs were able to sustain the expression markers of stem cells, such as CD29, CD44, CD73, CD90, CD105, and SSEA-4, as well as transcription factors that are critical in regulating pluripotency, such as Oct4-A, SOX2, and Nanog, after extended culturing in the medium. However, these cells do not express CD45 and CD133. Furthermore, we were also able to differentiate the propagated cells into different specific cell lineages, such as adipocytes and osteocytes after incubation with respective induction medium.
Conclusions: The study showed that PRIME-XV™ hAFSC Expansion Medium can be utilized effectively for routine hAFSCs expansion *in vitro.*

T-2047

E2F2 SILENCING INHIBITS EXPRESSION OF ONCOGENES AND SUPPRESSES PROLIFERATIVE CAPACITY OF HUMAN EMBRYONIC STEM CELLS

Daniela E. Suzuki, Adriana M. Nakahata, Oswaldo K. Okamoto
Department of Genetics and Evolutive Biology, Bioscience Institute - University of São Paulo, São Paulo, Brazil

Tumorigenicity of human embryonic stem cells (hESC) remains a major threat that limits their application in cell therapy protocols. Our group has identified hyperexpressed genes in cancer stem cells, among which *E2F2*, a gene involved in malignant transformation and stem cell self-renewal. Here we tested whether *E2F2* knock down would affect proliferative capacity of hESC *in vitro* and expression of oncogenes. A plasmid carrying shRNA and gentamicin resistance gene was transfected in H9 hESC to transiently silence *E2F2* expression. Transfected cells were selected based on gentamicin resistance after addition of 500µg.mL^{-1} of this antibiotic in the culture medium. A 57% knock down in target gene expression was obtained in hESC as determined by real-time PCR ($p<0.0001$). *E2F2* silencing significantly inhibited proliferation of hESC, indicated by a lower proportion of cells in S and G2 phases ($p<0.01$; $p<0.001$) and reduced capacity to generate hESC colonies (average size of 200-500 µm in diameter) after four days of culture ($p<0.05$), compared with control hESC transfected with scrambled shRNA. Furthermore, colonies generated by hESC with silenced *E2F2* were significantly smaller than those of control hESC. *E2F2* silencing also inhibited expression of the oncogenes *BMI1* and *HMGA* in hESC. These results suggest that *E2F2* knock down in hESC can reduce their proliferative capacity *in vitro*, providing a rationale for inhibition of hESC tumorigenicity based on transient silencing of specific key genes.

T-2048

NOVEL CELL SURFACE GLYCAN MARKERS FOR THE EARLY ISOLATION OF FULLY REPROGRAMMED HUMAN IPS CELLS

Milla Mikkola[1], Heli Mononen[1], Jere Weltner[1], Ras Trokovic[1], Virve Pitkanen[2], Juhani Saarinen[2], Tero Satomaa[2], Timo Otonkoski[1]
[1]Biomedicum Stem Cell Center, University of Helsinki, Helsinki, Finland, [2]Glykos Finland Ltd., Helsinki, Finland

Complex carbohydrate structures, glycans, cover all human cell surfaces, and individual glycan structures associated with stem cells (e.g. SSEA and Tra antigens) are commonly used to characterize pluripotent stem cells. The glycan structures emerging during pluripotent reprogramming, such as Tra-1-81, could be used to identify properly reprogrammed cells. Based on a global analysis of iPS cell glycans we aim to identify new markers that can enhance the early identification and purification of bona fide iPS cells.
In the present study, human foreskin fibroblasts were induced with Yamanaka factors using retroviruses. The glycan expression profile was studied at different time points after induction, showing that reprogramming is associated with marked changes in glycan structures, including up-regulation of pluripotency-associated large high-mannose and complex fucosylated N-glycan structures. Next, we used long-term imaging to track the fate of individual cells and found that specific markers, like Lewis blood group antigens become expressed at an early stage before the formation of true iPSC colonies. Finally, we FACS-purified cells based on the expression of candidate markers and studied their potential to form pluripotent stem cell colonies. The preliminary results suggest that cells with surface expression of the early stage glycan antigens have the potential to form pluripotent stem cell colonies.
Our results indicate that novel Lewis blood group glycan markers, in addition to the Tra antigen, enhance the detection of true iPS cells during the reprogramming process. At early time poins (day 8 -10) the percentage of these cells is low but increases rapidly by day 15 after induction. These findings may be applied in the development of more efficient and rapid methods to produce human iPS cell lines.

T-2051

SCALABLE EXPANSION OF HUMAN IPS CELLS IN STIRRED BIOREACTORS WITH THE OPTIMIZED E8 MEDIUM

Ying Wang[1], Bin-Kuan Chou[2], Sarah Dowey[2], Chaoxia He[2], Sharon Gerecht[1], Linzhao Cheng[2]
[1]Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, United States, [2]The Johns Hopkins University School of Medicine, Baltimore, MD, United States

Objectives: Production of a clinically relevant amount (usually considered as ~1 billion) of human induced pluripotent stem cells (hiPSCs) in chemically defined condition by practical and economic method remains a major challenge for advancing hiPSC technology from bench to clinic. Recent studies achieved significant improvements in serum-free culture medium and xeno-free substrates to enhance expansion efficiency of hiPSCs. Based on these achievements, a controllable and reproducible suspension culture system for in vitro expansion of hiPSCs will provide a feasible solution due to its scale-up capacity. Our goal was to establish a completely xeno-free suspension culture system in stirred bioreactor for large-scale expansion and cryopreservation of healthy and disease-specific hiPSCs.
Methods and Results: Two hiPSC lines derived from hematopoietic cells of either a healthy donor or a patient with sickle cell disease were first adapted from feeder-dependent to feeder-free adhesion culture and showed robust expansion to provide sufficient cells for the initiation of suspension culture. Cells cultured on E. Coli-expressed recombinant vitronectin (VNT) proteins were seeded as single cell suspension and formed homogenous aggregates with an average diameter less than 200 microns. The hiPSC aggregates showed normal metabolic activities and no compact center or local accumulation of apoptosis. The well-kept viability and stable expression of pluripotency markers indicated that the hiPSC cultured in E8-VNT condition rapidly adapted to suspension culture without the need of redundant step-by-step adaption. To determine the optimal operation requirements of the spinner flasks equipped with glass-ball impeller, we simulated the hydrodynamic properties including velocity field and shear stress by computational method. Indeed, under optimized agitating speed and split interval, high yield of 100 million hiPSCs was achieved in 60-ml working volume and in one single run of 3-day culture process. The two hiPSC lines were successively passaged in suspension for at least 10 passages without gaining abnormal karyotypes. The expansion rate was 2.8~3.3 folds per passage on average and the viability retained above 90%. Expression of pluripotency markers were kept higher than 98%, and the cells were able to spontaneously differentiated into cells from all three germ layers by in vitro and in vivo assay. The potential of directed hematopoietic differentiation was maintained at the same or even higher level than parallel adhesion cultures. Furthermore, we successfully achieved single-cell cryopreservation and the optimized recovery in E8-based serum-free freezing medium, which were also compliant to a scale-up strategy for hiPSCs in suspension, allowing a yield of ~1 billion cells within 20 days after thawing 1 million frozen cells.
Conclusion: We established a reproducible approach for rapid, economic, scalable and cGMP-compliant expansion of human iPSCs to meet the demand of practical research and clinical applications. Comparing to current suspension culture systems, ours shares the properties of high expansion rate, excellent maintenance of pluripotency, scalable capability, and yet advances in complete elimination of components from animal sources and remarkably cheaper than other xeno-free culture media.

T-2052

CHARACTERIZING SELF-RENEWAL OF HUMAN PLURIPOTENT STEM CELLS IN DYNAMIC SUSPENSION CULTURE

Daniel Nampe, Chad Beaudette, Ronak Joshi, Veronica Boulos, Chee Gee Liew, Hideaki Tsutsui
University of California, Riverside, Riverside, CA, United States

In order to produce clinically relevant number of cells, human Pluripotent Stem Cell (hPSC) culture needs a scalable platform. Alternative to conventional 2D adherent cultures, 3D suspension cultures are among one of the most promising methods for large-scale stem cell cultivation. Although static suspension culture have shown promising results in terms of growth and viability of hPSCs, a main concern for static suspension is the lack of control for culture outcomes (such as aggregate size and heterogeneity) and the cell's microenvironment. Consequently, we utilized an appropriate dynamic system, such as a conventional stirring vessel on suspended cells, to enable some control on the physical microenvironment. In our study, we applied different agitation rates, ranging from 0 rpm (static) to 120 rpm, on H9 human embryonic stem cell line that has been stably transfected with an OCT4-eGFP reporter, suspended in mTeSR media supplemented with 10 μM of ROCK inhibitor. After 7 days of culture in a stirring vessel, there was overall higher growth rate in 80-120 rpm (17-18 fold increase) than the static suspension (15 fold increase) and 20-60 rpm (1-7 fold increase). Furthermore, we observed that the median aggregate size decreased as agitation rate increased, indicating that intense fluid mixing either prevents or dissociates large cell

aggregates. Expression of key pluripotency markers was measured using flow cytometry and qPCR. The flow cytometry analysis showed that cells cultured under a range of 80-120 rpm agitation rate retained high levels of OCT4 and SSEA3 expression comparable to those found in the static condition. Interestingly, qPCR analysis showed that expression levels of OCT4 and NANOG under 80-120 rpm are significantly higher than under the static suspension. These results strongly indicate that fluid mixing affect growth and self-renewal of H9 cells either by modulating aggregate size or alternatively by inducing shear-induced mechanotransduction. For this reason, future work will decouple the aggregate size and shear forces and examine how these parameters impact short-term and long-term fate of hPSCs. Expected outcomes are identification of key physical parameters to control growth and self-renewal of hPSCs under dynamic suspension culture and a new design principle for stem cell culture vessels.

T-2053

HIGHLY-SPECIFIC MONOCLONAL ANTIBODIES AND KITS TO STUDY DNA HYDROXY-METHYLATION IN THE GENOME

Ignacio Mazon Pelaez, Sharon Squazzo
Diagenode, Denville, NJ, United States

In recent years, studies suggest that the mammalian genome undergoes various epigenetic modifications during development and embryonic stem cell (ESC) differentiation. Understanding the mechanisms that control these epigenetic signals is essential in deciphering stem cell biology and the patterns of gene expression during cellular differentiation. ESC gene expression relies heavily on transcriptional factor networks and epigenetic signals such as histone variants, histone modifications, DNA methylation, and non-protein-coding RNAs that give stem cells their ability to differentiate into different cell types.
5-methylcytosine (5-mC) is an important epigenetic modification involved in development and and cancer (in which this modification is frequently altered). 5-mC can be enzymatically converted to 5-hydroxymethylcytosine (5-hmC), and 5-hmC modifications are known to be prevalent in DNA of embryonic stem cells and neurons. Since 5-hmC is present in mammalian DNA at physiologically relevant levels and in a tissue-specific manner there is an important need to determine how 5-hmC can be distinguished from 5-mC and normal cytosine.
Here we present highly-specific monoclonal antibodies and kits for the differential study of the functions of 5-hmC, 5-mC, and unmodified C, with new data from Diagenode's hmC antibody, "hMeDIP" kits, and MeDIP kits. We provide data showing the specificity of 5-hmC vs. both 5-mC and unmodified C using dot blots, Dual MeDIP and hMeDIP. In situ cellular staining on interphase ES cells (data not shown) indicates distinct 5-hmC staining, both in overall brightness and in binding patterns, from that obtained with the Diagenode 5-mC antibody. Our data are consistent with distinct roles of 5-hmC and 5-mC. Our new kits and antibodies open the door to novel epigenetics studies and clarify the role of 5-hmC in differentiation, displacing methyl-binding proteins, regulating DNA repair, recruiting chromatin modifiers, and other important functions.

T-2054

NON-BIASED IMAGE PROCESSING METHODOLOGY FOR TYPING STEM CELLS BY THEIR MORPHOLOGY

Hiroto Sasaki[1], Taiki Tsuboi[1], Kei Kanie[2], Asuka Miwa[1], Yasujiro Kiyota[3], Hiroyuki Honda[1], Ryuji Kato[2]
[A]Graduate School of Engineering, [B]Graduate School of Pharmaceutical Sciences, [1]Nagoya University, Nagoya, Japan, [2]Nikon Corporation, Yurakucho, Tokyo, Japan

In clinical tissue engineering and cell therapy, although the cell is a "live material" with great variety and a highly sensitive nature, its production should be strictly controlled for safe and effective therapy. By the recent advances in stem cell culture technologies, cell banks which cover not only regular cell lines but also more specific "patient-specific cells" should expand with accumulating requirements both from the clinical and basic researches. To organize such cell banks maintaining various "delicate" types of cells, we consider novel technological development should be made to support routine cell maintenances.

Monitoring cellular behavior, such as adhesion, growth, and morphological features, is the only practical method, which is proven to be effective by the history, to check the status of cells in cell banks. Compared to other invasive methods, microscopic observation is known to provide many aspects of cellular status that lead to the speculation of cellular quality and purity with the best cost performance. However, at the same time, such decision based on observation and experiences are considered as “non-quantitative”, since monitoring images consisted huge biases and the experts’ decisions could never be described in objective style.
To break through the present tasks in cell maintenance system in cell banks, we here propose an image-based morphological typing for quantitate the experts’ feelings of cellular morphology, by combining automated cell image acquisition machinery together with cell image processing algorithms to eliminate the presently known biases of microscopic cell culture images. The image processing algorithm that we have developed, “IAWM (Image-auto wash method)”, is an application of chemical engineering particle analysis concept to eliminate the major image processing bias of “threshold-setting”. Our results indicate that not only normal human cell lines, but also mesenchymal stem cells could be typed with their morphologies effectively through our image acquisition and processing system. More than 10 types of cells and cell culture conditions could be categorized by cell morphologies quantitatively, indicating that the delicate experts’ decision making process in daily cellular monitoring could be modeled for automation of routine works.

T-2055

HUMAN BLOOD-DERIVED RAW MATERIAL: ENABLING CONTROLLED, CONSISTENT COLLECTION

William F. Busby[1], Thomas V. Ramos[1], Gaytha McPherson[1], Scott R. Burger[2]
[1]HemaCare Corporation, Van Nuys, CA, United States, [2]Advanced Cell & Gene Therapy, LLC, Chapel Hill, NC, United States

Human cells and tissue are critical raw materials for cell therapy and tissue-engineered products, as well as *ex vivo* gene therapy products. Quality of this living cellular raw material is a major determinant of final product characteristics, but inter-individual variability and biological heterogeneity are complicating factors. Controlled, qualified cell collection minimizes operational sources of variability, increasing likelihood of successful manufacturing. HemaCare, a long-standing supplier of human-derived blood components for novel biologic therapies and assays, medical devices, and clinical applications, established a program for controlling and qualifying its apheresis procedures and collection sites. This includes comprehensive staff training and qualification, documentation supporting cGMP operations, equipment and procedure validation, and monitoring in accordance with a rigorous quality system. Donor recruitment, screening and IRB-approved consents follow cGTP requirements. The quality program tracks and investigates deviations from process, complaints, and error rates in specific categories, representing donor screening and testing, collection, product preparation, testing, labeling, distribution, and cGMP/cGTP systems. As part of continuous process improvement, HemaCare validated or re-validated 14 apheresis instruments for mononuclear cell (MNC) collections at 3 collection sites, and validated the Horiba Pentra XL80 automated hematology analyzer. In addition, HemaCare has established in-house immunophenotyping by flow cytometry and immunomagnetic cell selection using the Miltenyi Biotec MACSQuant and AutoMACS. HemaCare performed 69,658 cellular apheresis collection procedures in the last five years, including collection of patient and normal-donor peripheral blood MNCs, G-CSF-mobilized peripheral blood progenitor cells, and plateletpheresis products, supporting commercial cell therapy and clinical trials, preclinical research, and validation studies. Capabilities are being expanded to include bone marrow, umbilical cord blood, and cord tissue collection, selection, and cryopreserved storage. Analysis of current HemaCare unmobilized apheresis products demonstrated consistently high MNC purity, with 93.8% of products containing ≥75% MNC, and an overall average of 85.2% MNC ± 6.6% (mean ± 1 SD), based on 174 sequential products measured with an automated hematology analyzer. These products displayed appropriately low red blood cell contamination, with a hematocrit averaging 1.8% ± 0.8% (mean ± 1 SD). In addition to controlled, consistent apheresis collection procedures, access to a pool of repeat donors also contributed to product consistency. Approximately 85% of HemaCare donors have donated apheresis products 5 or more times. MNC content of all HemaCare apheresis products had an average coefficient of variation of 7.7%, compared to 3.5% per donor. Rig-

orous operational controls, quality systems, and analytical capabilities enable optimal, consistent cell collection, a critical factor for successful research and development of novel cell-based products and technology.

T-2056

MESOANGIOBLASTS CONTRIBUTION TO DYSTROPHIN RESTORATION IN A HUMAN DMD IN VITRO MODEL

Elena Serena[1], Susi Zatti[1], Alice Zoso[1], Saverio Francesco Tedesco[2], Giulio Cossu[2], **Nicola Elvassore**[1]
[1]Department of Industrial Engineering, University of Padova, Padova, Italy, [2]Department of Cell and Developmental Biology, University College London, London, United Kingdom

The restoration of the protein dystrophin is the crucial end point of many research lines focused on the cure of muscular dystrophies. Several approaches are being developed, from cell therapy to exon skipping, and it emerges that a restoration of 20% of dystrophin could be sufficient to protect muscles from damages.
Our study aims at studying dystrophin restoration from human mesoangioblasts in an in vitro model of human skeletal muscles affected by Duchenne Muscular Dystrophy (DMD) recently developed in our laboratory. At the moment, mesoangioblasts are the best candidate for the treatment of muscular dystrophies because of their peculiar characteristics (such as being delivered in the arterial circulation) and they already entered the clinical trial phase.
Our in vitro model of DMD skeletal muscle is based on an engineered substrate with physiological stiffness and micropatterned parallel lanes of Matrigel. We exploited this model to investigate the capability of human mesoangioblasts in restoring dystrophin through the co-culture with primary DMD myoblasts (in a ratio of 1 to 9, respectively). Our model allowed the obtainment of fully differentiated myotubes composed of nuclei expressing dystrophin (mesoangioblasts and primary myoblasts) and dystrophic nuclei (DMD primary myoblasts).
The results obtained show that both primary myoblasts and mesoangioblasts are able to restore dystrophin in DMD myotubes. However, mesoangioblasts are more efficient than myoblasts in dystrophin production in terms of domain restoration and yield. The length of the domain expressing dystrophin is wider within mesoangioblast co-cultures: mesoangioblast sustain a domain ranging from 210 to 240 µm, while myoblast a domain from 40 to 100 µm. Indeed the amount of dystrophin produced in the mesoangioblast co-cultures is always higher. The quantification of western blot dystrophin bands show an average restoration of dystrophin of 15 % in myoblasts co-culture, while mesoangioblasts restore 40 % of dystrophin.
These results show that an in vitro model of human DMD skeletal muscle can be a valuable tool to investigate innovative therapeutic strategies. For instance, such a tool could be very helpful during a clinical trial: it could be used to characterize batches of cells and identify the most myogenic before in vivo implantation.

T-2057

MICROARRAY PLATFORM INDEPENDENT DETECTION OF SUBMICROSCOPIC AMPLICONS CRITICAL TO GENOMIC STABILITY IN HUMAN PLURIPOTENT STEM CELLS

Benjamin S. Nisler, **Seth M. Taapken**, Kimberly Leonhard, Erik McIntire, Karen Dyer Montgomery
Cytogenetics Department, WiCell Research Institute, Madison, WI, United States

Embryonic and induced human pluripotent stem cells acquire similar genetic abnormalities during culture. Many of these changes are visible by karyotyping, e.g. trisomy 12, but others are detectable only by higher resolution analysis. We compared copy number variants (CNVs) in three subclones of WA09 (H9) cultures across three microarray platforms (Agilent Sureprint G3 CGH+SNP [A], Illumina Human CytoSNP-12 [I], and NimbleGen Human CGH 385K and 135K WG-T [N]) regarding the ability to identify CNVs smaller than 2Mb. The overall frequency and type of CNV differs significantly between the platforms, primarily due to the reference design. Number and size of CNVs detected by platform follows: [A] =74-79 CNVs (1-95,845kb); [I] = 5-15 CNVs (31-95,676kb); [N] = 26-48 CNVs (51-132,249kb). Loss of heterozygosity was detected in all 3 subclones by both SNP microarrays: [A] = 1 region (4466-4483kb); [I] = 11-14 regions, (1072-2313kb).
These subclones are part of a series of cultures with related complex cytogenetic changes that occurred in H9 during culture. At early passage (p24) no abnormalities were found by karyotyping; by p33, the culture acquired an

inverted duplication of 12 short (p) arm and by p71, it progressed to a complex karyotype with two copies of the aberrant 12p, trisomy 14, and duplication of 17p. This progression was accompanied by an impressive increase in growth potential. Every karyotypic abnormality was seen in all three microarrays. Two submicroscopic aberrations with probable function were signaled in each platform: the long (q) arm amplicon of chromosome 20 at q11.21, thought to be related to growth advantage, and a 5q35.3 gain that may function as a cap to stabilize the 12p rearrangement.
We determined the minimal overlapping region of the 20q gain during routine testing of 235 human embryonic stem (hES) and induced pluripotent stem (iPS) cell cultures, 52 of which carried the duplication. The smallest amplicon was 285kb. Regardless of the size, the ID1 and BCL2L1 genes were always present. Based on these array findings, we designed a 123kb oligo fluorescence in situ hybridization (FISH) probe that includes both genes, to be used for rapid and inexpensive screening for the presence of the 20q amplicon.
We also compared microarray [N] to karyotype findings in early and late passage WA01 (H1) cell cultures. While many cultures were normal regardless of passage, a given culture, at passage 228, was karyotypically normal but carried the 20q11.21 amplicon (gp29275016-30575052), in addition to a submicroscopic loss in chromosome 2q33.2 (gp203637728-207137616). In another cell line, UC01, a recurrent acquired abnormality of 1q, dup(1)(q25q42.1), was found by karyotype. SNP microarray [I] analysis confirmed the presence of the 1q gain, and also detected the 20q11.21 amplicon.
The data we present supports the use of CGH and SNP technologies, in combination with targeted FISH analysis, to replace karyotyping as a monitor of genetic stability in stem cell cultures.

T-2058

MICROFABRICATED SCALE-DOWN DEVICE WITH ONLINE CONFLUENCY MONITORING FOR APPLICATIONS IN STEM CELL PROCESS DEVELOPMENT

Rhys J. Macown[1], Nicolas Jaccard[1], Alexandre Super[1], Lewis D. Griffin[2], Farlan S. Veraitch[1], **Nicolas Szita**[1]
[A]Biochemical Engineering, [B]Computer Science, [1]University College London (UCL), London, United Kingdom

Microfabricated bioreactors have been successfully applied to early bioprocess development in traditional biotechnology and typically offer significant reduction in reagent use, real-time monitoring and control of process variables, ease of sterilisation via disposable polymer technology, reduced labour due to automation, and the capability to rapidly test different processing conditions. Clearly, a microfabricated, adherent culture device that could translate these advantages to stem cell culture would be of great value to the fields of regenerative medicine and cell therapy where more insight into cell culture processes is needed. However, for the purposes of validation and scale up studies, a link must be maintained with conventional culture methods and potential production systems. Further, the device must be highly versatile to facilitate evaluation and optimisation of the wide range of process variables that affect stem cell fate. In particular, this versatility must extend beyond the control of the physicochemical properties of the culture medium to include auto/paracrine factors, growth surfaces, shear forces, and cell seeding densities. Finally, rapid online assessment of the product (cells) is crucial.
At UCL Biochemical Engineering, we have developed microfabricated devices and monitoring concepts that have the potential to address these needs. We have realized a novel, autoclavable, microfabricated culture device, with a re-sealable culture chamber. This re-sealable culture chamber allows traditional static seeding in an otherwise fully assembled device. The device reversibly seals with a TC-PS microscope slide (or any other standard sized slide), allowing the use of typical growth surfaces. We demonstrate the potential of the device by showing results for feeder-attached human embryonic stem cell (hESC) colonies, and mouse embryonic stem cells (mESC).
To accelerate stem cell bioprocessing, there is clearly a need for a quantitative method for online characterisation of adherent cell cultures in general and that of co-cultures in particular. Such an online characterisation method will allow accurate and reproducible measurement of the effect of changes in experimental conditions (e.g. culture substrate and ECM used, medium formulation). Combining the exquisite control over the fluid flow that microfabricated / microfluidic devices offer with these novel concept of online monitoring will enable dynamic control of the culture conditions. To this effect, we have started to tailor image-processing concepts towards the label-free detection and characterisation of hESC colonies co-cultured with iMEF feeder cells, and of mESCs. In this present-

ation, we will present these novel microfabrication and monitoring concepts, how they are combined over one single platform, and how this will lead to real-time monitoring of culture progress in the future. Additionally, we will show first results of real-time detection of bulk and peri-cellular dissolved oxygen concentrations, and of a fully automated parallelised platform that fits on a microscope stage which will permit real-time data-rich experimentation for stem cell process development.

T-2061

IN VITRO AND IN VIVO ANALYSIS OF MGHA/COLLAGEN-BASED SCAFFOLD ENGINEERED WITH OVINE AMNIOTIC FLUID MESENCHIMAL CELLS.

Stefano Tetè[1], **Alessandro De Carlo**[1], Vincenzo Luca Zizzari[2], Umberto Di Tore[1], Felice Lorusso[1], Luca Valbonetti[3], Luisa Salini[4], Mauro Mattioli[5], Enrico Gherlone[6]

[A]Department of Medical, Oral and Biotechnological Sciences, [B]Department of Pharmacy, [1]University "G. D'Annunzio" Chieti-Pescara, Chieti, Italy, [2]Department of Biomedical Sciences, University of Teramo, Teramo, Italy, [3]Department of Dentistry, DBS Francavilla Al Mare, Chieti, Italy, [4]Department of Biomedical Sciences, University of Teramo, Chieti, Italy, [5]Oral Science, University "Ateneo Vita-Salute San Raffaele", Milan, Italy

Objectives: The present study was designed to evaluate the regenerative influences of mesenchimal sheep amniotic fluid (oAFMCs) seeded on a synthetic scaffold. The synthetic scaffold was engineered in vitro with oAFMCs and were grafted into the maxillary sinuses of adult sheep before evaluating the occurrence and progression of bone regeneration at different times.
Materials and methods: oAFMCs previously induced in osteogenic differentiation, were marked with a fluorescent marker and sown on the scaffold. oAFMC were screened by flow cytometry in order to evaluate the expression of haemopoietic markers, of adhesion molecules, of stemness intracellular markers and MHC class I and II antigens. The plasticity of oAFMCs to differentiate into bone cell lineage was assessed on samples of thawed oAFMCs by using validated cultural conditions. Six adult sheep underwent bilaterally maxillary sinus augmentation. One sinus filled with two engineered blocks of biomaterial with oAFCMs (test side) and the other only with biomaterial (control side). The biomaterial used has a tridimensional structure, constituted by a combination of tipe I collagen and HA enriched with magnesium. The scaffold is commercially available as RegenOss®. Animals were divided randomly into two groups and sacrificed after 45 and 90 days. Then, block sections of sinus were obtained and were performed radiographical micro-CT analysis. Morfometric analysis allowed to evaluate the total area covered by new formed bone, residual biomaterial and total vascular area at the two experimental times. Fluorescent microscopy allowed the evaluation of marked oAFMCs during the bone healing period. Cells were analyzed for evaluating the effects of the expansions and cryopreservation processes, the presence of hematopoietic markers, adhesion molecules, stemness markers and MHC I and II markers.
Results: The cells isolated from amniotic fluid appeared as an uniform population of fusiform cells after 3 *in vitro* passages. The oAFMCs expanded *in vitro,* after cryopreservation, did not modify their molecular profile. The RegenOss® scaffold showed a high ability to support oAFMC adhesion and proliferation. After three days of incubation the scaffolds were, in fact, completely covered by alive oAFMCs. After 45 days regenerated sites with the engineered scaffold alone resulted completely surrounded from connective tissue, not yet mineralized but highly vascularized. At 90 days, in both sinus the scaffold appeared resorbed. In all samples, the large amount of mineralization zones of the extracellular matrix, appeared distributed at the scaffold periphery, in contact with maxillary sinus walls, highlighting a close integration between bone and scaffold. After cryopreservation, oAFMCs did not seem to modify their molecular profile and resulted to have the ability of differentiating in osteogenic way.
Conclusions: This study demonstrates the high regenerative potential of oAFMCs and their plasticity. The use of oAFMCs increased bone deposition and a more rapid angiogenic reaction thus probably supporting the higher proliferation index recorded. RegenOss® grafted into maxillary sinus displays a clear osteoinductive effect that may be significantly improved by the presence of oAFMCs. The scaffold seems an adaptive filler of contenitive bone de-

fects. Results confirmed that mesenchimal amniotic fluid cells may be able to improve clinical performances of biomaterial. This study was partly funded by PRIN 20102ZLNJ5.

T-2062

MAMMARY EPITHELIAL CELL LINEAGE TRACING USING INTRADUCTAL ADENOVIRUS INJECTION

Luwei Tao
Department of Medicine, Brigham and Women's hospital, Boston, MA, United States

Lineage tracing experiments (such as those based on the Cre/lox approach) have provided invaluable information of mammary epithelial hierarchy under the physiological setting. However, further application of this approach to the study of in vivo mammary epithelial cell (MEC) hierarchy is limited by the availability of inducible CreERT-expressing mouse lines. Recently, we developed a new technique of lineage tracing based on intraductal injection of Cre-expressing adenovirus to murine mammary glands (MGs). Successful intraductal injection can be performed in mice of 3-weeks of age to adults. In pilot experiments, by injecting CMV-Cre adenovirus into MGs of the Rosa26YFP conditional Cre-reporter mice, we found that both luminal and basal MECs can be labeled (by turning on the YFP reporter), which ensured unbiased study of both MEC lineages using this approach. Meanwhile, the labeling efficiency could be titrated by changing the viral titer, which ensured proper clonal analysis. Since adenovirus does not integrate into genome, injection of adenovirus only results in transient expression of Cre recombinase. By injecting CMV-GFP adenovirus and by following the GFP signal in the injected MGs, we found that the percentage of GFP+ cells reached to the peak level 3 days after injection, then quickly dropped one week later and completely disappeared 2 weeks after injection. So far several mammary epithelial lineage-specific Cre-expressing adenoviruses, including K8-nlsCre (nlsCre: Cre recombinase with a nuclear localization signal), K14-nlsCre and WAP-nlsCre, have been generated based on their corresponding well-characterized promoters. K8-nlsCre and K14-nlsCre adenoviruses specifically labeled luminal and basal MECs, respectively. The lineage specificity was maintained after long-term chase for at least 1.5 months, which was in agreement with a previous study demonstrating that both luminal and basal lineages were maintained by their corresponding unipotent lineage-specific mammary stem cells in vivo. These two viruses are currently used for chasing the cellular origin of mammary tumors in multiple oncogenic mouse models. In contrast, WAP-nlsCre labeled a small population of luminal cells with progenitor features (Lin-CD24+CD29loCD61+CD14+C-kit+). Interestingly, the WAP-nlsCre-labeled MECs were maintained during long-term chase even after pregnancy and lactation. Previous studies using WAP-Cre transgenic mice suggested that WAP-Cre-marked MECs in virgin mice might represent a unique population of alveolar progenitor cells. Our data further suggested that the population of MECs marked by WAP-nlsCre adenovirus might represent a unique luminal restricted stem cell population in adult MGs. In summary, our novel adenovirus-based lineage tracing approach is comparable to the CreERT inducible mouse system in achieving sensitive labeling with high specificity. It enables in vivo lineage tracing for the study of both normal MEC development and of the cellular origin of breast cancer quickly, and has the potential to become a powerful genetic tool to screen new lineage markers or pathway-specific reporters for normal MECs and mammary tumor cells.

T-2063

DEVELOPMENT OF STEM CELL CULTURE PLATFORM WITH PATTERNED CONDUCTIVITY FOR ELECTRICAL CONTROL OF CELL BEHAVIOR

Lin Wang, Jianli Zou, Li Liu, Ken-ichiro Kamei, Franklin Kim
Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Kyoto, Japan

The extracellular matrix (ECM) plays a pivotal role in providing proper microenvironment for stem cell adhesion, growth, differentiation, and tissue formation. Thus creating an ECM-like microenvironment in vitro has long been a widely accepted paradigm for the design of cell culture scaffold. Currently, the great majority of biomimetic scaffold development and modification research has focused on engineering scaffold materials with biomimetic chemical, mechanical, or topological features, yet the incorporation of in vivo-like electrical properties has been largely

ignored. Endogenous direct-current electric fields with specific spatial distribution universally exist in the extracellular spaces of developing and regenerating tissues. Such patterned electric fields often serve as electrical cues in guiding cell migration or proliferation. Hence, being able to recapitulating ECM electrical properties in a cell culture scaffold would render researchers a new dimension of control in directing stem cell fate.
In this study, a biocompatible graphene oxide (GO) thin film with patterned conductivity is developed as a conductive stem cell culture platform. Such platform is capable of generating controlled voltage gradients and electric fields within cell culture by tuning the resistivity pattern of the GO film, which enables researchers to direct stem cell migration and differentiation electrically. GO nanosheets were synthesized by Hummer's method and assembled into 2D film on top of glass via polyelectrolyte complexation between negatively charged GO nanosheets and positively charged chitosan which serves as a glue between GO and glass. The conductivity of GO film is patterned by photolithography of exposing GO film to UV light through a photomask, and further adjusted by reducing GO via heat treatment. UV irradiation followed by heat treatment resulted in reduced GO film with resistivity around ~2 kΩ·cm, which is comparable to the resistivity of soft tissue (~1 kΩ•cm). While the area blocked by photomask has resistivity of ~ 0.7 MΩ·cm, which is 300 times higher than the area exposed to UV light. Biocompatibility test of culturing human induced pluripotency stem (hiPS) cells on the surface of GO and reduced GO surfaces coated with Matrigel for 7 days showed above 90% viability, which is comparable to traditional cell culture treated polystyrene (PS) surfaces. Cells cultured on the GO and reduced GO surfaces exhibited significantly higher degree of cell adhesion and proliferation in comparison with PS surfaces. When cultured in mTeSR1, cells are able to maintain their pluripotency to the same level as cells on PS surfaces. hiPS cell adhesion, proliferation, and maintenance of their pluripotency is independent with respect to GO reduction state, suggesting that the change of cell behavior on GO with patterned conductivity will be only resulted from the pattern of substrate conductivity or electric fields.
Our data collectively demonstrated that we have successfully created a biocompatible scaffold with patterned conductivity for stem cell culture. It provides an ideal platform for the studies of endogenous electrical cues in directing stem cell growth and differentiation. In the future this platform will be tested in the utility of directing stem cell differentiation towards neuron cells or cardiomyocytes, which are known to be sensitive to electrical stimuli.

T-2064

CRYOPRESERVED OVINE AMNIOTIC EPITHELIAL CELLS ENHANCE REGENERATIVE RESPONSE OF A CUSTOM MADE SCAFFOLDS: AN IN VITRO AND IN VIVO STUDY.

Stefano Tetè[1], **Vincenzo Luca Zizzari**[2], Umberto Di Tore[1], Alessandro De Carlo[1], Felice Lorusso[1], Luisa Salini[3], Aurelio Muttini[4], Barbara Barboni[4], Adriano Piattelli[1]

[A]Department of Medical, Oral and Biotechnological Sciences, [B]Department of Drug Sciences, [1]University "G. D'Annunzio" Chieti-Pescara, Chieti, Italy, [2]Department of Dentistry, DBS Francavilla Al Mare, Chieti, Italy, [3]Department of Biomedical Sciences, University of Teramo, Teramo, Italy

Objectives: The aim of this study was to evaluate, by the in vitro and in vivo, the effect of Ovine Amniotic Epithelial Cells (oAECs) addiction, to a custom-made scaffold.
Materials and methods: oAECs were previously obtained by enzymatic digestion from amnios of sheep slaughtered at 3 months of pregnancy. oAECs were tested with FACS analysis and stored in liquid nitrogen before using in preclinical studies. Cryopreserved vials of oAECs were randomly thawed to test: cytokeratin 8, α-SMA, MHC, hemapoietic and adhesion and pluripotent markers, ability to differentiate into three different mesodermal cell lineages. The syntetic scaffold was produced by a 3D software with CAD / CAM and realized with a mixture of 30% HA, 60% β-TCP and 10% α-TCP throught a direct rapid prototyping (DRP). The scaffold was tested in vitro by seeding oAEC and assessing the growth rate, the gene expression, the growth and signs of cell. Subsequently, six adult sheep underwent bilaterally maxillary sinus augmentation. One side was treated with biomaterial blocks with oAECs (test side), while other side with blocks of the biomaterial alone (control side). The animals were divided randomly into two groups and sacrificed at 45 and 90 days after surgery in order to assess the tissue regeneration.
Results: Once expanded in vitro for 3 consecutive passages, these cells do not appear to modify their molecular profile, as confirmed by FACS and intracellular content of cytokeratin 8/α-SMA. In detail, the cells oAECs cryop-

reserved and expanded showed no hematopoietic markers as well as MHC class II antigens. On the contrary, the oAECs maintain a stable positive for markers of pluripotency such as TERT, SOX2, NANOG and OCT4. The RT-PCR analysis showed a stable profile of expression of four mesodermal genes COL1, SCXB, OCN and LPL. The oAECs grown in culture medium produce an extended osteogenic mineralization of the extracellular matrix as shown by Alizarin Red staining and gene expression. High cell density has been recorded on the surface outside and inside the channels of the scaffold after 3 days of dinamic incubation. In vivo, our micro-CT results show the integrity of the bone with newly formed mineralized tissue bridges that after 90 days depart from the periphery of the scaffold to the walls of the breast. In side test after 45 days the surgical access is already healed, the Schneider membrane shows no signs of inflammation, and it is possible to locate an abundant extracellular matrix deposition in continuity with the endogenous bone structure, index of an occurred graft integration. On the contrary, in the control side, the surgical access appears still open after 45 days, with poor deposition of extracellular matrix infiltrated by fibroblasts and inflammatory cells, with hyperplasia of Schneider membrane, phenomena attributable to those of "a foreign body reaction."
Conclusions: The oAECs transplantation supported an overall increase of bone deposition either directly contributing to osteogenesis or indirectly modulating the major mechanisms (inflammation, cellularity and angiogenesis) involved in tissue regeneration. The present results provide the first preclinical evidence in favor of an efficient and safe therapeutic role of oAECs. These results support the hypothesis that oAECs holds much promise for the development of cell-based therapies in craniofacial surgical applications leading to the idea of a their safe use under allotransplantation settings. This study was partly funded by PRIN 20102ZLNJ5.

T-2071

EFFECTS OF SIMULATED MICROGRAVITY ON OLIGODENDROCYTE PROLIFERATION AND LINEAGE PROGRESSION: IMPLICATIONS FOR CENTRAL NERVOUS SYSTEM REPAIR

Araceli Espinosa-Jeffrey, Pablo Paez, Shalini Kumar, Alana Taniguchi, Megumi Hirose, Vilma Spreuer, Jean de Vellis
UCLA, Los Angeles, CA, United States

Myelination of nerve fibers is one of the most significant milestones of vertebrate evolution as it is responsible for rapid and efficient conduction of electrochemical messages to and from the central nervous system CNS. Myelin is produced by oligodendrocytes in CNS any abnormality in OL or in myelin itself produces severe pathologies of the CNS during development or in neurodegenerative disorders where myelin regeneration is difficult to achieve. In many cases OLP fail to mature and therefore they can't re-myelinate axons in dys- and demyelinating diseases such as Pelizaeus Merzbacher's disease (PMD), multiple sclerosis (MS) or after traumatic injury. Moreover, white matter damage (WMD) is a leading and increasing cause of functional and cognitive disability, and is detected in a significant percentage of preterm human infants. Our overall goal is to investigate the prospects of cell replacement therapies for patients that require normal OL and myelin to restore proper CNS function. Enhanced remyelination through transplantation of OL may offer a realistic approach to restoring meaningful neurological function. Yet, effective methods to generate OL progenitors (OLP) that would be devoid of other cell populations prior to be grafted, still need to be developed. In the late 70s, our laboratory pioneered the culture of rat OL progenitors in significant amounts for quantitative biochemical studies and it is still the method of choice. More recently we have designed developmental stage specific proprietary culture media that allow for the modulation of proliferation and differentiation of OL in culture. During the past three years we have established a culture system to study the impact of simulated microgravity produced by a 3D-clinostat robot (Mitsubishi Heavy Industries) on glial cell cultures. We subjected OLP to short exposure of sim-μG, we labeled proliferating OLP from control and 0G treated cultures with the thymidine analogue bromo-deoxyuridine (BrdU) over a period of three days. Twenty four hour pulses of 10μM BrdU were given at 0h (immediately before starting the 0G treatment), 24h and 48h. After each BrdU pulse, proliferating progenitors were identified by double immunofluorescence for BrdU and NG2 and the relative number of $NG2^+/BrdU^+$ cells was quantified in each cell population. Our results demonstrate that rodent and human OLP display enhanced and sustained proliferation when exposed to sim-μG without interfering with their lineage progression, specific markers expression or maturation. **Innovation 1:** Currently there is no cure for myelin disorders despite the increasing prevalence and its clinical importance. To our knowledge, the use of neither *real* nor

simulated microgravity to increase the production of OL in adequate numbers has been described. **Innovation 2:** Oligodendrocytes possess intrinsic plasticity that can be modulated by the environment, an understanding on the plasticity of OL in simulated μG will provide us with an innovative platform to test novel pharmacological and cell replacement therapies to counteract myelin deficiency. Grant Support: National Multiple Sclerosis Society grant #PP1498; Center grant NIH #04612.

T-2072

DYNAMIC PHOTOCONTROL OF CELL SIGNALING THROUGH INDUCIBLE PROTEIN CLUSTERING

Lukasz J. Bugaj[1], Atri Choksi[1], Colin Mesuda[2], Ravi Kane[4], David Schaffer[3]
[A]Bioengineering, [B]Chemical and Biomolecular Engineering, [C]Chemical and Biological Engineering, [1]University of California Berkeley, Berkeley, CA, United States, [2]Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY, United States

Stem cell fate decisions result from dynamic regulation of numerous cell signaling pathways, whereby a cell can choose divergent fates based on the strength, timing, or location of even an individual protein signal. However, the chemical and genetic tools widely used to probe cell signaling suffer from poor spatiotemporal resolution and cannot easily recapitulate the dynamics with which protein signals affect cell fate choices, thus limiting our ability to understand fundamental stem cell maintenance and differentiation mechanisms. Optogenetic techniques have recently enabled rapid and reversible cellular protein activation through light inducible protein heterodimerization, homodimerization, and gene transcription within living cells. Protein oligomerization, however, represents a distinct and important mode of activation for numerous cell signaling events, yet its study and control remains challenging due to the lack of tools to inducibly modulate a protein's oligomeric state
Here we report a novel genetically encoded protein platform for modular and tunable control of protein homo-oligomerization in response to blue light. We show this oligomerization to be rapid, reversible, and repeatable, and we demonstrate its utility in modulating the activity of diverse signaling pathways within mammalian cells. Specifically, we used the light-induced clustering of a fusion protein to induce strong β-catenin signaling in both 293Ts and neural stem cells, achieving a higher transcriptional response than the natural Wnt3a ligand. Light control of β-catenin may be particularly useful in stem cell and developmental studies, where spatial and temporal gradients of β-catenin activation regulate cell fate and tissue morphogenesis. We further demonstrate modularity of light inducible clustering as a means of dynamic intracellular protein control by clustering and activation of the RhoGTPases Rac1, RhoA, and Cdc42. This result not only allows access to photocontrol of these factors, but it also suggests clustering as a heretofore unknown mode of activation for this important family of proteins and highlights the potential of the clustering module for discovering protein oligomerization as a novel activator of signaling events.
The development of light-induced protein oligomerization in mammalian cells presents a valuable addition to the optogenetic toolbox. The Cry2 system exhibits on and off rates on the order of seconds and minutes, respectively, enabling the interrogation of network responses to precisely controlled time-varying signaling inputs. The spatial distribution of these signals can be defined with high resolution, enabling researchers to easily pattern signaling onto a field of cells, or even within a single cell. Importantly, our successful photoactivation of the β-catenin, Rac1, and RhoA pathways suggests the ability to abstract Cry2-mCherry as a "plug-and-play" clustering module. This modularity promises to rapidly expand the optogenetic toolbox and in the future endow numerous other signaling pathways with photocontrol, provided a clustering event regulates a node of the signaling network.

T-2073

A NEW DEFINED, CONSISTENT SERUM REPLACEMENT FOR THE SUPPORT OF HUMAN PLURIPOTENT STEM CELLS

Hiroko Yokoe, Arazdordi Toumadje, Helen Josephson, Rosemarie Garr, **Jonathan M. Auerbach**
GlobalStem, Rockville, MD, United States

Traditionally, fetal bovine serum has been used as an additive to cell culture media for the in vitro growth of pluripotent stem cells. Fetal bovine serum is an inherently variable component of the cell culture system so consistent

performance demands extensive screening of manufactured lots. To circumvent this problem, companies began offering “stem cell qualified” serum. One issue with this solution is that the qualification is not always performed on a stem cell line relevant to the researchers own cell lines. Another solution lies in the development of serum-free supplements designed to completely replace the serum in the culture medium. This can sometimes be a costly path forward. Here, we report the development of a new, defined, cost-effective serum replacement - PluriQ SR - for the growth of human pluripotent stem cells. We have cultured a human ESC line for 20 passages in PluriQ SR and found that it remains undifferentiated and karyotypically stable. The new serum replacement is suitable for human ESC and iPSC growth in co-culture with MEF and under feeder-free growth conditions. In addition, consistent product performance is obtained by minimizing inconsistencies in separately manufactured lots.

T-2074

SUSTAINED LEVELS OF FGF2 MAINTAIN UNDIFFERENTIATED STEM CELL CULTURES WITH BIWEEKLY FEEDING

Steven Lotz[1], Susan Goderie[1], Nicolas Tokas[1], Sarah Hirsch[1], Barbara Corneo[1], Sheila Le[1], Akhilesh Banerjee[2], Ravi Kane[2], Jeffrey Stern[1], Sally Temple[1], **Christopher Fasano**[1]
[1]Neural Stem Cell Institute, Rensselaer, NY, United States, [2]Rensselaer Polytechnic Institute, Troy, NY, United States

An essential aspect of stem cell culture is the successful maintenance of the undifferentiated state. Many types of stem cells are FGF2 dependent, and pluripotent stem cells are maintained by replacing FGF2-containing media daily, while tissue-specific stem cells are typically fed every 3rd day. Frequent feeding, however, results in significant variation in growth factor levels due to FGF2 instability, which limits effective maintenance due to spontaneous differentiation. We report that stabilization of FGF2 levels using controlled release PLGA microspheres improves expression of stem cell markers, increases stem cell numbers and decreases spontaneous differentiation. The controlled release FGF2 additive reduces the frequency of media changes needed to maintain stem cell cultures, so that human embryonic stem cells and induced pluripotent stem cells can be maintained successfully with biweekly feedings.

T-2081

UBIQUITOUS CHROMATIN OPENING ELEMENTS (UCOE) PREVENT EPIGENETIC TRANSGENE SILENCING IN MURINE AND HUMAN PLURIPOTENT STEM CELLS AND THEREOF DERIVED TISSUES

Mania Ackermann[1], Nico Lachmann[1], Nils Pfaff[1], Hartung Susann[2], Reto Eggenschwiler[3], Christian Brendel[5], Manuel Grez[5], Axel Schambach[4], Robert Zweigerdt[2], Tobias Cantz[3], Thomas Moritz[1]
[A]REBIRTH RG Reprogramming, [B]Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), [C]REBIRTH RG Stem Cell Biology, [D]Institute of Experimental Hematology, [1]Hannover Medical School, Hannover, Germany, [2]Institute for Biomedical Research, Georg-Speyer-Haus, Frankfurt, Germany

Introduction: Pluripotent stem cells (PSC) represent a promising target population for gene therapy approaches. However, epigenetic transgene silencing, especially during the differentiation of PSCs, constitutes a major obstacle for this strategy. One way to overcome this problem is the use of ubiquitous chromatin opening elements (UCOE). We recently demonstrated that the defined 1.5 kb A2UCOE derived from the human heterogenous nuclear riboprotein A2/B1/ chromobox homolog 3 (HNRPA2B1/CBX3)- locus used in combination with the truncated elongation factor-1α (EFS) promoter effectively prevents CpG-methylation-associated silencing of lentiviral transgene expression during hematopoietic differentiation of murine (m)PSC. Based on these results we here investigate the transgene promoting effect of the A2UCOE (i) in combination with other (viral) promoters, (ii) during differentiation into cells of other germ layer such as hepatic (endoderm) and neuronal (ectoderm) cells, as well as (iii) in human (h)PSCs and their differentiated progeny.
Methods/Results: (i) Also when combined with the spleen focus forming virus (SFFV) promoter the A2UCOE effectively stabilized transgene expression in murine iPSCs (~80% vs. ~3% dTomato+ in passage (p)3) as well as thereof differentiated CD41+ hematopoietic progenitor cells (~80% vs. 1-3% dTomato+ cells). Similar to the EFS pro-

moter, bisulfite sequencing revealed protection of the SFFV promoter from CpG-methylation to be associated with this effect. (ii) To analyse transgene stabilization by the A2UCOE during hepatic and neurogenic differentiation the physiological EFS promoter was utilized. Similar to the effect observed during hematopoietic differentiation, the A2UCOE effectively reduced silencing of the EFS-promoter and allowed for sustained transgene expression in ~97% of hepatically (EFS-driven controls: ~3%) and ~65% of neuronally (EFS-driven controls: ~3%) differentiated cells, respectively. (iii) When we investigated transgene expression in human (h)PSCs profound silencing of EFS-driven transgene expression was observed in undifferentiated H9 human embryonic stem cells (hESCs). This was prevented by the A2UCOE (~23% vs. 83% dTomato+ cells in p6, respectively). Again, the effect was associated with reduced EFS promoter methlyation in A2UCOE transduced cells (~33% vs. 90% methylated CpGs). Similar efficiency of the A2UCOE was noted in non-differentiated hES3 hESCs and human induced pluripotent stem cells (hiPSCs) derived from CD34+ hematopoietic stem/progenitor cells. Furthermore, analysis of hES3 cells during non-directed differentiation revealed sustained, A2UCOE-mediated transgene expression in ~80% of Tra-1-60 negative progeny cells harvested on day 13 (EFS-driven controls ~10%). Even more important, similar effects were observed during the directed differentiation of hPSCs into cardiomyocytes or monocytes/macrophages.
Discussion: We here prove efficacy of the A2UCOE in murine PSC when combined with viral and physiological promoter elements as well as during differentiation into cells of all three germlayers. In addition we demonstrate the A2UCOE to stabilize transgene expression in human PSC in the undifferentiated status and during mesodermal differentiation. Thus our data introduce UCOEs as a generalized concept to stabilize transgene expression during the generation of PSC-derived transgenic cell therapy products.

T-2082

DIRECTED EVOLUTION OF ADENO-ASSOCIATED VIRUS FOR ENHANCED GENE DELIVERY TO PLURIPOTENT AND ADULT NEURAL STEM CELLS

Melissa A. Bartel, David V. Schaffer
Chemical and Biomolecular Engineering, University of California, Berkeley, Berkeley, CA, United States

Stem cells are characterized by their ability to self-renew and to generate one or more mature cell types. Both pluripotent stem cells (PSCs) and neural stem cells (NSCs) present attractive targets in gene therapy and/or cell replacement therapies to treat nervous system injury or disease. Unfortunately, most gene delivery vectors are not capable of targeted gene delivery to stem cells. Adeno-associated virus (AAV) is a promising gene delivery vector that has been used in nearly 100 clinical trials to date. We have applied directed evolution, a high-throughput molecular engineering approach, to create AAV variants capable of efficient and specific transduction of human pluripotent stem cells or adult neural stem cells. Here, we report the *in vitro* and *in vivo* infection properties of our novel AAV variants and demonstrate the variants' utility for genetic engineering of stem cells.
Following a directed evolution approach consisting of one round of evolution and three selection steps on human embryonic stem cells, a variant of AAV2 was isolated and found to mediate increased transduction of several human embryonic stem cell (hESC) and induced pluripotent stem cell (iPSC) lines. In addition, the variant was capable of gene-targeting frequencies of up to 0.1%, approximately 10-fold higher than the targeting frequencies in pluripotent stem cells obtained using naturally occurring AAV serotypes. Furthermore, using zinc finger nucleases in conjunction with AAV-mediated gene targeting, this variant achieved gene-targeting frequencies of 1.2%, a >10-fold increase in the already efficient AAV-mediated gene targeting in hESCs. This capability can enable the use of hPSCs for *in vitro* studies of disease mechanisms, developmental processes, and drug discovery and toxicity.
Furthermore, following a directed evolution approach consisting of three rounds of evolution and ten selection steps on adult rat NSCs, a variant containing a peptide insertion was previously isolated and found to mediate a 50-fold increase in the transduction of rat NSCs. The variant also exhibited increased transduction of murine NSCs, human fetal NSCs, and human embryonic stem cell-derived neural progenitor cells *in vitro*. Moreover, the variant was capable of gene-targeting frequencies of up to 0.16%, approximately 5-fold higher than the targeting frequencies in rat NSCs obtained using naturally occurring AAV serotypes. To build upon the *in vitro* success of this variant, the *in vivo* infection properties of this novel AAV variant were analyzed. It was capable of efficient and selective transduction of rat and mouse neural stem cells in the adult hippocampus compared to several natural AAV sero-

types. This variant therefore has strong potential for manipulating the genome of adult NSCs *in situ* for biological or therapeutic applications.

T-2083

A STRATEGY FOR REPRODUCIBLE MEASUREMENTS OF ANTIBODY-BASED DETERMINATION OF STEM CELL POTENCY

Greg Cooksey[1], Guokai Chen[2], Jeanette Beers[2], Anne L. Plant[1], John T. Elliott[1]

[1]Biosystems and Biomaterials Division, NIST, Gaithersburg, MD, United States, [2]National Heart, Lung, and Blood Institute; iPSC and Genome Engineering Core Facility, NIH, Bethesda, MD, United States

The spontaneous reprogramming of adult cells to induced pluripotent stem cells (iPSCs) creates new opportunities for cell therapies and disease-related studies. However, characterization of these lines is often ambiguous. We are developing methods and protocols that will allow unambiguous day-to-day and lab-to-lab comparisons of antibody-based measurements of pluripotency markers in iPSC cultures. Because microscopy and flow cytometry are typically used to obtain information about stem cell surface markers in a qualitative, rather than quantitative, manner, it is difficult to evaluate whether two laboratories are achieving the same or different responses for the same marker. This ambiguity results in questions about the reliability of markers as indicators of pluripotency, and about the robustness of laboratory protocols for culturing cells. This measurement challenge is being addressed by employing a robust cell-labeling strategy that allows immunofluorescence staining on cells to be normalized to total cellular protein. We demonstrate a protocol that uses commercially available maleimido fluorophores to covalently label cellular proteins through free cysteine groups within cells, and we show that this approach can provide a reliable internal fluorescence reference for normalizing fluorescent antibody labeling of cellular markers. Using these robust protocols we are generating reference data for pluripotent markers for the ND2.0 iPSC line (Chen et al., Nature Methods, 8(5), 424-431). ND2.0 is a feeder-free and integration-free line which is being disseminated by NIH CRM as a candidate reference line for comparison to new iPSC lines and for protocol validation. Our results show that referencing the intensity of AlexaFluor488-labeled TRA-1-60 and other antibodies to a maleimide label provides a means of intensity normalization and allows comparison of staining intensities across different microscope configurations.

T-2084

PROBING OF THE ROLE OF EXTRACELLULAR MATRIX IN THE MAINTENANCE OF PLURIPOTENT STEM CELL PLURIPOTENCY

Robert E. Schwartz[1], David Braga Malta[1], Nathan Reticker-Flynn[1], Kelly R. Stevens[1], J. M. S. Cabral[2], C, Lobato da Silva[2], Gregory Underhill[1], Sangeeta Bhatia[1]

[1]Harvard–MIT Division of HST, Massachusetts Institute of Technology, Cambridge, MA, United States, [2]Instituto Superior Tecnico, Portugal, Lisbon, Portugal

Human embryonic and induced pluripotent stem cells (hESC/hiPSC) have the ability to differentiate into all the cell lineages and thus hold great promise for the treatment of human disease. However, current popular methods to grow hiPSC require mitotically inactivated feeder cells (MEFs) or undefined extracellular matrix (ECM) mixes (ie. Matrigel) and thus introduce animal factors and lot variability. To identify human native or recombinant ECM and understand the role of ECM in the maintenance of pluripotency, we developed an expanded ECM microarray (μECM) platform1. This platform enables the parallel analysis of the effects of 741 unique environments, comprising all of the single and pairwise combinations of 38 distinct ECM, within a single microscope slide. We identified at least three ECM combinations that support iPSC self-renewal and pluripotency for more than 50 passages (>85%oct3/4+tra1-60+ssea1+). ECM expanded iPSC maintained normal karyotype, the potential to form embryoid bodies in vitro and teratomas in vivo and to differentiate towards the cardiomyocyte, hepatic, and neuronal lineages in vitro. Varying experiments showed the dependence of pluripotency on specific ECM combinations: (i) single matrix molecules are unable to maintain the pluripotency phenotype (ii) blocking ECM proteins and signaling induces loss of pluripotency. Our results suggest that ECM proteins when presented in specific combinations are a reliable and defined platform to support pluripotent stem cells. Moreover, this defined platform has enabled the exploration of the role of integrin signaling in stem cell maintenance implicating downstream signaling pathways in the regulation of pluripotent gene networks.

T-2091

HIGH-CONTENT SCREENING FOR PLURIPOTENCY REGULATORS USING DISTRIBUTION-BASED SINGLE-CELL ANALYSIS

Julian Gingold[1], Ed Coakley[2], Jie Su[1], Dung-Fang Lee[1], Zerlina Lau[1], Hongwei Zhou[1], Christoph Schaniel[1], Dan Felsenfeld[1], Ihor Lemischka[1]

[1]Mount Sinai School of Medicine, New York, NY, United States, [2]Program in Applied Mathematics, Yale University, New Haven, CT, United States

High-content screening has become a popular tool in experimental biology to study the effects of a large number of compounds or conditions on a biological system, facilitating a fine-grained cell-level characterization of changes following treatment to a large number of samples. Several studies on embryonic stem cells (ESC) have used screens to identified pluripotency regulators, primarily using transgenic fluorescent reporters driven by the pluripotency gene Pou5f1. Most currently used approaches reduce the effect of a treatment to a single parameter such as median or mean cell fluorescence. In doing so, a significant amount of potentially informative data from the high-resolution images of treated cells, each with distinct levels of reporter expression, is typically disregarded. Therefore, a more comprehensive method for the analysis of screens at the single-cell level and with minimal data assumptions is desired.
To more effectively identify outlier “hit” conditions in a screen that affect the reporter on the cell population and not necessarily the population median, we developed a novel, highly generalizable approach for analyzing large-scale data in which each condition is described by a set of single-cell-level parameters and applied it to a genome-wide short inhibitory (si) RNA screen for effects on pluripotency using a Nanog promoter-driven GFP reporter mouse ESC line. By considering each set of cell parameters as a single sample from a distribution, we can find a basis to best capture the information distance across condition effects. We apply this minimal basis to cluster and visualize the range of biological and technical effects. We distinguish GFP-reducing effects of siGFP and siSox2 controls with far greater accuracy than conventional median-cell analysis and identify numerous outlier genes with significant effects that would have been missed by other methods. Among these outliers, we validate the effect of Chek1 on the pluripotent state. Collectively, our approach demonstrates the successful application of distribution-based hit identification in screens and has led to the identification of numerous pluripotency regulators.

T-2092

CONTRASTING HUMAN AND MOUSE EMBRYONIC STEM CELL SELF-RENEWAL USING CELL-TYPE-SPECIFIC PREDICTIVE NETWORKS

Karen G. Dowell[1], Allen K. Simons[2], Zack Z. Wang[4], Kyuson Yun[3], Matthew A. Hibbs[5]

[1]Graduate School of Biomedical Sciences and Engineering, University of Maine, Orono, ME, United States, [A]Computational Services, [B]Research, [2]The Jackson Laboratory, Bar Harbor, ME, United States, [3]Division of Hematology, Johns Hopkins Medicine, Baltimore, MD, United States, [4]Department of Computer Science, Trinity University, San Antonio, TX, United States

The molecular mechanisms that drive stem cell self-renewal in different classes of stem cells are diverse and only partially understood. Here, we clarify the molecular foundations of mouse and human embryonic stem cell (ESC) self-renewal by ap-

plying a proven Bayesian network machine learning approach to integrate cell-type-specific, high-throughput data collected from dozens of studies, under hundreds of conditions. In contrast to prior computational efforts in this field, we focus on predicting functional linkages among genes and proteins in the context of biological processes known to be active in a single cell type by carefully tailoring the data compendium, manually curating the training gold standard, and rigorously analyzing both computational performance and the biological meaning of contextual predictions. For this study, we assembled separate data compendiums for mouse and human ESCs. We then integrated these data into separate predictive networks focused on biological processes associated with ESC self-renewal and cell fate determination to identify conserved and divergent associations among genes and proteins. Computational evaluations, literature validation, and signaling pathway analyses show that our results are highly accurate and biologically relevant. Our mouse and human self-renewal networks, available online at www.StemSight.org, predict many novel players currently being experimentally validated. They serve as the foundation for future pluripotent stem cell and cancer stem-like cell studies and can be used by stem cell researchers to explore hypotheses about gene function in the context of self-renewal and to prioritize genes of interest for experimental validation.

T-2093

MAPPING THE HEMATOPOIETIC HIERARCHY BY SINGLE CELL ANALYSIS OF THE CELL SURFACE REPERTOIRE

Guoji Guo[1], Sidinh Luc[1], Eugenio Marco[3], Ta-Wei Lin[2], Keyong Zou[4], Guo-Cheng Yuan[3], Stuart H. Orkin[5]
[A]Hematology/Oncology, [B]Molecular Genetics Core Facility, [1]Children's Hospital Boston, Boston, MA, United States, [2]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA, United States, [3]Boston Open Labs, Cambridge, MA, United States, [4]Harvard Stem Cell Institute, Cambridge, MA, United States

Stem cell differentiation pathways are most often studied at the population level, whereas the critical decisions are executed at the level of single cells. We have established a highly multiplexed, quantitative PCR assay to profile in an unbiased manner a panel of all commonly used cell surface markers (280 genes) from individual cells. Using the methodology, we analyzed over 1500 single cells throughout the mouse hematopoietic system. The comprehensive single cell dataset permits mapping of the mouse hematopoietic stem cell (HSC) differentiation hierarchy by computational lineage progression analysis. We identify CD55 as an early megakaryocytic and erythroid lineage (MegE) marker that separates both common myeloid progenitors (CMP) and multipotent progenitors (MPP) into functionally distinct subgroups, suggesting early branching of the MegE lineage differentiation. In the most primitive hematopoietic stem cells, an HSC transcription factor module positively regulates MegE lineage priming, which marks the first molecular evidence of initial lineage separation in the hematopoietic hierarchy. The strategy has broad applicability in other stem cell systems.

iPS Cells

T-2101

THE SHIFTING CONCEPTS OF IDENTIFIABILITY AND PRIVACY IN STEM CELL GENOMICS: SCIENTIFIC, ETHICAL AND POLICY IMPLICATIONS.

Rosario Isasi[1], Bartha M. Knoppers[1], Peter W. Andrews[2], Glyn Stacey[3]
[1]Centre of Genomics and Policy, McGill Univ, Montreal, QC, Canada, [2]Department of Biomedical Science, University of Sheffield, Sheffield, United Kingdom, [3]UK Stem Cell Bank, National Institute for Biological Standards & Control, Potters Bar, United Kingdom

Increasingly,
methods and associated tools allowing for individual identification in publicly
accessible databases (e.g. genomic, ancestry, etc.) are being developed. While
the generalizability of such methods continues to be evaluated, and corresponding
evidence-based risk re-assessments debated; there is no doubt that the concepts

of identifiability and privacy are shifting, and so, the expectations of stakeholders (scientists, research participants, regulatory and funding bodies together with policy-makers). In the context of stem cell genomics, particularly with respect to the derivation of pluripotent stem cell lines (e.g. iPSCs), few studies have focused on the probability that similar methods could be applied to deduce donor identity and ensuing implications. *(International Stem Cell Forum Ethics Working Party, "Publishing SNP Genotypes of Human Embryonic Stem Cell Lines (hESC): A Policy Statement". Stem Cell Reviews and Reports, 2011).*This presentation will assess the strengths and weaknesses surrounding current technical and ethical safeguards aimed at protecting iPSC donor identity. To that end, we will prospectively analyze what constitute an acceptable threshold for identifiability and privacy protections. Finally, we will propose a balanced approach between the goals of open science and data sharing with the respect for fundamental ethical principles such as autonomy and privacy.

T-2102

CLONAL GENETIC AND HEMATOPOIETIC HETEROGENEITY AMONG HUMAN INDUCED PLURIPOTENT STEM CELL LINES

Jason A. Mills[1], Prasuna Paluru[1], Lei Ying[1], Gustavo Mostoslavsky[4], Darrell N. Kotton[4], Deborah L. French[2], Mitchell J. Weiss[3], Paul Gadue[1]

[A]Center for Cellular and Molecular Therapeutics (CCMT), [B]Center for Cellular and Molecular Therapeutics, [C]Division of Hematology, [1]The Children's Hospital of Philadelphia, Philadelphia, PA, United States, [2]Department of Medicine and Center for Regenerative Medicine (CReM), Boston University and Boston Medical Center, Boston, MA, United States

Induced pluripotent stem cells hold great promise for modeling human hematopoietic diseases. However, intrinsic variability in the capacities of different iPSC lines for hematopoietic development complicates comparative studies and is currently unexplained. We created and analyzed three separate iPSC clones from the fibroblasts of three different normal individuals and have designated these lines, CHOPWT1, CHOPWT2, BMC. These iPSC lines were created by using a standardized approach that included excision of integrated reprogramming genes by Cre-Lox mediated recombination. All nine iPSC clones expressed standard pluripotency markers, generated teratomas, and contained normal karyotypes. Gene expression profiling assays were performed on the nine iPSC clones and compared to three human embryonic stem cell (ESC) lines. To reduce variability caused by low levels of spontaneous differentiation in the ESC and iPSC cultures, FACS was used to isolate cells expressing similar levels of the pluripotency markers SSEA3 and TRA-1-81 prior to gene expression profiling. In addition to gene expression profiling, the hematopoietic potential of all lines was compared. Both gene expression profiling and hematopoietic differentiation assays showed that independent lines from the same individual were generally more similar to each other than those from different individuals. However, one iPSC clone (WT2.1) exhibited distinctly different gene expression, proliferation rate and hematopoietic developmental potential relative to the other iPSC lines. This "outlier" clone also acquired extensive copy number variations during reprogramming, which may be responsible for its divergent properties. Our data indicate how inherent and acquired genetic differences can influence iPSC properties, including hematopoietic potential. Our study also highlights the inherent reproducibility in hematopoietic development potential of iPSC clones derived from a particular patient source and reinforces the importance of carefully characterizing iPSC lines to avoid artifactual effects on iPSC differentiation most likely induced by mutations acquired during the reprogramming process.

T-2103

INTEGRATION PREFERENCES OF LENTIVIRAL VECTORS IN INDUCED PLURIPOTENT STEM CELLS

Luiza C. Junqueira-Reis[1], Virgínia Picanço-Castro[3], Fabiana F. Bressan[4], Lílian F. Moreira[5], Marcela C. De Freitas[5], Dimas T. Covas[6], Elisa M S Russo[2]

[1]Pharmaceutical Sciences College of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Brazil, [B]Department of Clinical Analysis Toxicological and Bromatological, [1]Pharmaceutical Sciences College of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Brazil, [2]Blood Center of Ribeirão Preto - USP, Ribeirao Preto, Brazil, [3]College of Animal Science and Food Engineering, University of Sao Paulo, Ribeirao Preto, Brazil, [A]Department of Medical Clinic, [4]Medicine College of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Brazil

The induced pluripotent stem cells (iPSC) emerged with the promise of circumvent some of the limitations in the use of embryonic stem cells (ESC), like ethical issues, biological safety, imune compatibility and availability. This cells can be generated from somatic cells of healthy individuals or from patients with some genetic disease, making then an important tool for drug screening, construction of disease models and toxicological trials. Great advances have happened in reprogramming differentiated cells through the forced exogenous expression of transcription factors (TF), mostly by lentiviral vectors (LV), which provide an efficient reprogramming. However, the lentiviral insertion in the human genome and its influence in reprogramming are not well known. In this work, we evaluate the insertion profile of LV used to generate human iPS cells. The iPSC were generated, by our group, from human fibroblasts transduced by LV containing 3 TF [SOX2, TCL-1A and C-MYC (iPS TSM)], and from mesenchymal cells derived from human adipose tissue transduced by a polycistronic LV containing 4 TF [OCT4, SOX2, KLF4 and C-MYC (iPS OSKM)]. Five isolated colonies of each iPS cell were mapped and analyzed for the insertion sites through LM-PCR technique. The digested genomic DNA was amplified with a primer for the viral LTR e another for a synthetic linker. The products were cloned, sequenced and analyzed in database to identify similarities with the human genome, among other analyzes. The insertions were distributed on all chromosomes, but the statistical analysis showed preference for the 16, 17 and 20 for the iPS TSM, and for the 11, 15 and 17 for the iPSC OSKM. Both vectors showed preference (p < 0,0001) for genic region, with about 50% of the integrations occurring in these regions in iPS TSM, and with around 45% of integrations reaching genes in iPSC OSKM; more than 90% of these integrations are in introns for both cells. Regarding to the affected genes by the integration, we found a group of genes, 3 in each cell line, whose products form a group of proteins related to cell division and chromosomic distribution, and these insertions could be related to reprogramming due to participation of it proteins in cell proliferation. We found a group of genes related to the regulation pathway of actin cytoskeleton, and an insertion in this region could affect this pathway, even the insertion occurring in intronic region. We identify two affected genes which are related to susceptibility to prostate cancer AR (androgen receptor), related with development and maintenance of male sexual characteristics, and with female fertility, and MXI1 (MAX interactor 1) whose product inhibit the transcriptional activity of C-MYC, known oncogene used in iPSC protocols, strongly regulated in normal cells and often deregulated in human cancers. Thus, these insertions could be related to reprogramming, and an insertional mutation in the tumor suppressor gene MXI1 could also lead to a malignant phenotype by deregulation of C-MYC. Summarizing, this work shows that the integration sites may contribute to the reprogramming, and, despite possible negative effects of integration, these iPSC are still an important tool for in vitro studies. Even if integrative systems are not used in future clinical applications, identify factors that influence the selection of insertion site is important for determination of “safe” chromosomal regions for the integration, increasing the safe in clinical use.

T-2104

WHOLE EXOME SEQUENCING REVEALS 228 CONSERVED MUTATIONS IN PARENTAL AND THREE LRRK2 PARKINSON'S PATIENT-DERIVED IPSC LINES

Dezhong Yin, Yukari Tokuyama, Anna DiRienzo, Jeanmarie Curley, Kevin Grady
ATCC, Gaithersburg, MD, United States

Parkinson's disease is the second most common neurodegenerative disorder traditionally characterized by the selective loss of dopaminergic neurons in the substancia nigra of the midbrain. Patient-specific induced pluripotent stem cells (iPSCs) provide an opportunity to model human disease in relevant cell types. To provide a better research tool for studying Parkinson's disease, we generated three iPSC lines from dermal fibroblasts of a patient with point mutations in Leucine-Rich Repeat Kinase 2 (LRRK2) gene, the most common cause of familial Parkinson's disease, using three different reprogramming methods: Retroviral, Sendai viral, or episomal expression of *OCT3/4, SOX2, KLF4,* and *MYC* genes. The Parkinson's iPSC lines generated with different reprogramming methods demonstrated similar cell morphology, pluripotent marker expression, and the ability to differentiate into three germ layers. Compared to a hiPSC line-derived from a healthy subject, these Parkinson's iPSC lines showed a similar efficiency of neural differentiation into neural progenitors from iPSC-derived embryoid bodies. To more effectively model Parkinson's disease, we sequenced exons of all three Parkinson iPSC lines by exome sequencing with an Agilent's SureSelect 51 Mb array and compared to the parental exome sequencing data. Regardless of the reprogramming methods, all three iPSC lines retained the parental LRRK2 gene mutations at positions 50 (R50H), 723 (I723V), and 2397 (M2397T), which have previously been reported in Parkinson's disease patients. Compared to the hg19 human genome reference, the parental fibroblast and all 3 iPSC lines have over 300 genes with missense mutations and there are 228 genes with missense mutations conserved among all four cell types. Therefore by using integrating or non-integrating reprogramming methods, we have created three fully characterized iPSC lines that carry LRRK2 mutations, which will allow us to better investigate the underlying mechanisms of Parkinson's disease in a disease-relevant cell type, as well as to understand the potential differences observed by the reprogramming methods.

T-2105

STUDYING GENETIC DIVERSITY AND EVOLUTION BY CELLULAR REPROGRAMMING

Iñigo Narvaiza[1], Maria C. N. Marchetto[1], Ahmet M Denli[1], Christopher Benner[1], Thomas Lazzarini[1], Apua CM Paquola[1], Gene W Yeo[2], Alysson R Muotri[3], Fred H Gage[1]

[1]The Salk Institute, La Jolla, CA, United States, [2]Department of Cellular and Molecular Medicine, Stem Cell Program, University of California San Diego, Institute for Genomic Medicine., La Jolla, CA, United States, [3]Dpt. of Pediatrics/ Rady Children's Hospital San Diego, Dpt. of Cellular & Molecular Medicine, University of California San Diego, School of Medicine, Sanford Consortium, La Jolla, CA, United States

Despite their substantial genomic similarity, humans and non-human primates (NHP) differ considerably in terms of brain function, cultural complexity and language acquisition. Understanding cellular and molecular differences between us, humans, and our closest living relatives, chimpanzees (Pan troglodytes) and bonobos (Pan paniscus), is essential to the basic comprehension of the evolution and diversity of our own species. Until now, preserved tissues have been the main source of most comparative studies between humans and non-human primates. However, these tissue samples do not fairly represent the distinctive traits of live cell behavior, are not amenable to genetic manipulation and do not allow translation of observed differences into phenotypical divergence. We hypothesized that induced pluripotent stem cells (iPSCs) could provide a unique biological resource to elucidate relevant phenotypical differences between human and the great apes and that those differences could have potential adaptation and speciation value. In this study, we describe the generation and characterization of iPSCs from chimpanzees and bonobos as novel tools to explore our most recent evolution. Non-human primate iPSC behave similarly to human cells regarding self-renew capacity and pluripotency potential. Comparative RNA-seq expression analysis of human and NHP iPSCs revealed differences in regulation of Long Interspersed Nuclear Element (LINE-1 or L1) transposons. A force involved in shaping mammalian evolution, L1 elements are retrotransposons that have remained active during primate evolution. We observed decreased levels of L1 restricting factors in NHP iPSCs, which was correlated with an increased L1 mobility and L1 mRNA expression. In addition, we found increased copy numbers of species-specific L1 elements in the genome of chimpanzees compared to humans, supporting the idea that increased L1 mobility in NHPs is not limited to iPSCs in culture and may have also occurred in the germline during primate evolution. We propose that differences in L1 mobility may have differentially shaped the genomes of humans and NHPs

and could have had an adaptive significance. In this work we also present a new perspective on the use of iPSC technology. We reprogrammed somatic cells from humans and from our closest living relatives, with the goal of generating a powerful tool for the study of early stages of development and possible validation of evolutionary genomic and transcriptomic modifications with practical implications to human health.

T-2106

PARP1 AND TET2 MEDIATE EPIGENETIC EVENTS IN THE EARLY STAGE OF IPSC REPROGRAMMING

Claudia A. Doege[1], Keiichi Inoue[1], Skylar Travis[1], David B. Rhee[1], Ryousuke Fujita[1], Paolo Guarnieri[2], Toru Yamashita[1], Alan Shih[3], Ross L. Levine[3], Emily I. Chen[4], Asa Abeliovich[1]

[A]Pathology, [B]Biomedical Informatics Shared Resources, [1]Columbia University, New York, NY, United States, [2]Human Oncology & Pathogenesis Program, MSKCC, New York, NY, United States, [3]Proteomics Center, Stony Brook University, Stony Brook, NY, United States

Introduction
Somatic
cell reprogramming has great promise for regenerative medicine. However, understanding the basic mechanisms of reprogramming appears to be crucial for improvement of the iPSC technology
toward clinical applications in humans.
So far only little is known about the epigenetic events in the early
phase of iPSC generation. Here, we
identify two epigenetic regulators, Parp1 and Tet2, which mediate the early epigenetic
events at endogenous pluripotency loci.
Methods
To
define the early epigenetic changes during iPSC reprogramming we employed time
course analyses of gene expression, transcription factor and histone binding
via chromatin immunoprecipitation (ChIP), and DNA methylation
measurements. For the latter we used *Hpa*II digestion and *Msp*I digestion of glucosylated DNA allowing the distinction between
the DNA methylation species 5-methylcytosine (5mC) and 5-hydroxymethylcytosine
(5hmC). Candidate epigenetic regulators
were identified via proteomic analysis of nuclear fractions of fibroblasts and
pluripotent cells. Proteins which were
abundant in pluripotent cells and absent or very low in fibroblasts were tested
for their capacity to promote iPSC generation.
Subsequent studies examined if and how these epigenetic regulators
modulate the early epigenetic events in iPSC reprogramming.
Results
We
identified two epigenetic regulators, Parp1 and Tet2, which work in concert
with the reprogramming factors to mediate the early epigenetic events in iPSC
reprogramming. They are recruited to the
endogenous pluripotency loci (e.g., *Nanog*,
Esrrb) in the early phase of iPSC
reprogramming, preceding transcription of these loci. While Parp1 functions in the regulation of 5mC,
Tet2 is essential for the generation of 5hmC. Parp1 and Tet2 are needed to establish the
histone modifications that typify an activated chromatin state, and Parp1
promotes accessibility to the Oct4 reprogramming factor.
Discussion

These
data support necessary but distinct roles for Parp1 and Tet2 in the regulation
of epigenetic marks and local chromatin structure at pluripotency loci during
an early stage of iPSC reprogramming that precedes transcription at these loci.
The data further suggest that 5hmC does
not simply represent an intermediate in the 5mC demethylation process, but
functions as an epigenetic mark, possibly recruiting *trans*-acting factors that promote chromatin remodeling.

T-2107

DEVELOPING AN RNA SIGNATURE TO PREDICT IPSC DIFFERENTIATION POTENTIAL

Paige Cundiff[1], Caroline Hendry[1], Sunita D'Souza[1], Gaurav Pandey[2], Ana Sevilla[1], Ivan Carcamo-Orive[3], Hope Lancero[4], Fahim Abbasi[4], Thomas Quertermous[4], Joshua W. Knowles[3], Ihor Lemischka[1], Eric Schadt[2]
[A]Developmental and Regenerative Biology, Black Family Stem Cell Institute, [B]Institute for Genomics and Multiscale Biology, [1]Mount Sinai School of Medicine, New York, NY, United States, [A]Cardiovascular Institute, [B]Cardiovascular Medicine, [2]Stanford School of Medicine, Stanford, CA, United States

Induced pluripotent stem cell (iPSC) technology has been shown to be useful for the creation of model systems to study Mendelian conditions where the genetic susceptibility is due to single gene defects with large effects. In contrast, for common conditions where the genetic basis is more complex, the use of induced pluripotent stem cell (iPSC) approaches presents particular challenges. The stochastic variability in iPSC clones threatens the signal to noise ratio that could make it difficult to gain insight into the genetic architecture of these conditions. It is our hypothesis that some of the difficulties in dissecting the genetic basis of insulin resistance can be overcome through the study of a large number of iPSC lines using a systems biology approach. As part of the NHLBIs "NextGen" Consortium effort we are creating a large number of iPSC lines from a large number of individuals (several lines each from ~ 300 subjects) with a well-defined phenotype ("clamp" measure of insulin resistance) and genome wide association data. The goals of the project include: 1. Development of efficient methods of iPSC production and characterization necessary for very large numbers of iPSC lines; 2. Creation of a "shared resource" of iPSC lines through cooperation with a national biobank; 3. Differentiation of iPSC lines to the cell types of interest (adipose and endothelial cells) as model systems for insulin resistance. A major question at the outset of the project was whether peripheral blood mononuclear cells (PBMCs) or fibroblasts would be most amenable for reprogramming for this type of endeavor. Another inherent difficulty in the creation of so many iPSC lines is the cost in time and money to create and maintain multiple clones from each individual to a stage when differentiation is possible. To gain insight into these questions and to develop more streamlined approaches to iPSC production and differentiation we directly compared Sendai virus-based reprogramming in PBMCs and fibroblasts. We then used various protocols to differentiate the iPSC clones (~ 6 per condition) to mesenchymal progenitors (MPCs), which are a precursor to adipose cell differentiation. By applying RNASeq methodology at an early iPSC stage (passage 6 post reprogramming) we are seeking to develop an RNA "signature" that can predict the ability of each individual clone to progress to the MPC stage. In preliminary experiments including ~ 20 iPSC clones, we used a logistic aggression approach on RNASeq data to develop a classifier that achieved > 85% accuracy in predicting which iPSC clones would differentiate into "good" versus "bad" MPCs as defined by typical morphology and confirmed by FACS. We are presently validating this classifier using a much larger number of iPSC clones. If the results are confirmed we will be able to save substantial time, energy and money by allowing us to limit the number of iPSC clones that we have to maintain for long periods of time.

T-2108

NOVEL MARKERS TO DISTINGUISH PLURIPOTENT AND NONPLURIPOTENT CELLS

Rene H. Quintanilla, Joanna Asprer, Uma Lakshmipathy
Primary and Stem Cell Systems, Life Technologies, Carlsbad, CA, United States

A steep challenge in stem cell research is the identification and characterization of cells. This issue is amplified during the process of somatic reprogramming, a long and complex process that lasts for several weeks. It is important to identify and pick the right colonies for further expansion and characterization. Commonly, surface antibodies against pluripotent specific markers such as SSEA4, Tra-1-60 and Tra-1-81 are used but this method is expensive and sterility is a concern. Differential expression of Alkaline Phosphatase (AP) in pluripotent cells is a useful tool but most available AP substrates are toxic to the cells and once stained cannot be propagated further.
We have earlier reported the development of a novel Alkaline Phosphatase Live Stain (AP Live Stain) that specifically stains pluripotent stem cells while preserving cell integrity. More recently, using differentially expressed markers identified from transcriptome comparison of parental fibroblasts, partially and fully reprogrammed cells, we have identified several differential surface markers. One surface marker was highly expressed in parental fibroblast cells and partially reprogrammed cells but absent in embryonic stem cells and fully reprogrammed induced pluripotent stem cells. This marker was successfully used in combination with pluripotent markers to show distinct expression patterns between fully reprogrammed pluripotent colonies and partially or non-reprogrammed colonies during reprogramming. Antibody against the surface marker was utilized for rapid enrichment of pluripotent stem cells from parental fibroblast during reprogramming and from feeder murine fibroblasts in feeder-dependent culture systems. Gene expression pattern of a focused set of pluripotent and differentiation genes indicate a signature comparable to pluripotent stem cells harvested using traditional enzymatic and feeder-free culture methods. Identification of a combination of positive and negative markers will enable easier detection and early identification of true iPSC colonies with extended utility in isolation and enrichment.

T-2111

BROAD T-CELL RECEPTOR REPERTOIRE IN T LYMPHOCYTES DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Chia-Wei Chang[1], Yi-Shin Lai[1], Lawrence S. Lamb[2], Tim M. Townes[1]
[A]Department of Biochemistry and Molecular Genetics, [B]Department of Medicine, [1]University of Alabama at Birmingham, Birmingham, AL, United States

Human induced pluripotent stem cells (hiPSCs) have considerable potential for the treatment of inherited and acquired disorders. Recently, antigen-specific T lymphocytes derived from hiPSCs have been reported. However, T lymphocyte populations with broad T cell receptor (TCR) diversity have not been generated. We report that hiPSCs derived from skin biopsy are capable of producing T lymphocyte populations with a broad TCR repertoire. In vitro T cell differentiation follows a similar developmental program as observed in vivo, indicated by sequential expression of CD7, intracellular CD3 and surface CD3. The γδ TCR locus is rearranged first and is followed by rearrangement of the αβ locus. Both γδ and αβ T cells display a diverse TCR repertoire. Upon activation, the cells express CD25, CD69, cytokines (TNF-α, IFN-γ, IL-2) and cytolytic proteins (Perforin and Granzyme-B). These results suggest that most, if not all, mechanisms required to generate functional T cells with a broad TCR repertoire are intact in our in vitro differentiation protocol. These data provide a foundation for production of patient-specific T cells for the treatment of acquired or inherited immune disorders and for cancer immunotherapy.

T-2112

EPIGENETIC REGULATION OF HOXA LOCUS IN INDUCED PLURIPOTENT STEM-LIKE CELLS FROM HUMAN GASTRIC CANCER CELLS LINES

Shin-Wei Wang[1], Chuang-Jung Liu[1], Sophie Shen-Wen Wang[1], Shigeo Saito[3], Yukio Nakamura[4], Kazushige K. Yokoyama[3], Deng-Chyang Wu[2]
[1]Koahsiung Medical University Hospital, Kaohsiung, Taiwan, [B]Gastroenterology, [1]Koahsiung Medical University Hospital, Kaohsiung, Taiwan, [2]Koahsiung Medical University, Kaohsiung, Taiwan, [3]RIKEN BRC, Tsukuba, Japan

Human gastric cell line, KMU-CS12 (CS12) and from an immortal cell line, KMU-CSN (CSN), which were derived from putative human gastric stem cell/progenitor cell clone, KMU-GI2. CS12 expressed cancer cell phenotypes, i.e. the ability of anchorage-independent growth high frequency (44%) and to the expression of Oct4, a stemness marker and many types of cancer cells, and tumor development in immune deficient mice. Chromosomal analysis showed a characteristic duplication of the short arm of chromosome 7 to chromosome 12. Agilent Human 1A oligo-array analysis and qPCR revealed that homeobox genes like Hoxa9 (57.83 fold), Hoxa7 (32.01 fold), Hoxa4 (24.14 fold), Hoxa5 (7.24 fold) and Hoxa13 (6.14 fold) were highly expressed in CS12 cells. Induced pluripotent stem cells (iPSCs) were generated by electroporation using AP1 transcription factor Jun Dimerization protein 2 (JDP2) and Oct4 in both cell lines. JDP2 plays roles in cell cycle regulation, cellular senescence, nuclear reprogramming and oncogenesis through the epigenetic control involved in cascades of $p19^{Arf}$-Mdm2-p53-p21-cyclin/CDK or $p16^{Ink4a}$-cyclin/CDK-RB-E2F. iPSCs expressed three standard stemness genes like Oct4, Sox2 and Nanog, but parental CS12 and CSN did not show the alkaline phosphatase activity and the staining of anti-SSEA-3, -4, Tra-1-60 and Tra-1-81. CS12 induced the tumor formation in SCID mice but CS12 iPSCs repressed the tumor progression. These results indicate that iPSCs might function as possible tumor repressor. Moreover, the significant reduced expression of Hoxa 4, 5, 7 and 9 and the enhanced expression of Hoxa13 were detected in CS12iPSCs. The expression of histone H3K27me3 was enhanced and H3K18Ac was repressed in CS12iPSCs compared with CS12. The expressions of $p16^{Ink4a}$ and polycomb repressive complex subunits Bmi1 and Ezh2 were also repressed in CS12iPSCs. Thus, we conclude that the epigenetic alterations are one of the critical factors for inactivation of CS12 cancer phenotypes and cancer development. We also discuss the role of HOXA genes in nuclear reprogramming and cancer development in gastric cell line.

T-2113

A MODIFIED METHOD FOR IMPLANTATION OF INDUCED PLURIPOTENT STEM CELLS UNDER THE RODENT KIDNEY CAPSULE

Fuli Zhu[1], Bin Sun[1], Yan Wen[1], Renee Reijo Pera[2], Bertha Chen[1]

[A]Department of Obstetrics/Gynecology, [B]Department of Obstetrics/Gynecology; Institute for Stem Cell Biology & Regenerative Medicine, [1]Stanford University School of Medicine, Stanford, CA, United States

INTRODUCTION

A key safety concern in the implementation of induced pluripotent stem cell (iPSC)-based cell therapy in humans is the potential for tumor formation caused by the presence of trace pluripotent iPSCs within the transplanted cells. The standard teratoma assay is commonly used to assess the *in vivo* malignant potential of these stem cell impurities in the transplanted cells. The incidence of teratoma formation varies depending on the injection site in the rodent. Kidney capsule is one of the most efficient teratoma formation sites for iPSCs and/or human embryonic cells because the capsule minimizes cell migration after transplant. However, a substantial amount of transplanted cells can leak out of the incision during the transplantation of cells into the kidney capsule, making it difficult to ascertain the initial cell count. To minimize this source of error, cells are often mixed with grafts and then implanted with the graft. While this methodology helps sequester the cells, it is difficult to determine an accurate cell count, and there is an additional variability due to the graft material. These issues introduce uncertainty in studies targeted at investigating malignant potential of stem cell-derived transplants.

OBJECTIVE

To enable single cell transplant into the kidney capsule with minimal cell leakage using a modified surgical transplantation method

METHODS

Preparation of glass capillary pipettes in our modified method for kidney capsule injection is essential. The glass capillary pipettes are pulled on one end with forceps

while heated on flame to make a long, fine, but not very sharp tip. After exposing the kidney outside of the abdominal cavity, we transplant single iPSCs with the slender tip into the kidney capsule. The transplant is done on the upper and lateral side of the kidney in order to avoid damage to major blood vessels. The glass tip is slowly pushed

into the capsule, as far as possible, towards the inferior pole of the kidney to avoid perforating the kidney capsule in other areas. After injection, the fine tip is pulled out of kidney capsule slowly to avoid cell leakage.

RESULTS

The modified surgical procedure is easy to perform, and the single cell suspension can be deposited under the kidney capsule without leakage. Lack of leakage was confirmed with Trypan blue. Up to 40 µl of cell volume can be deposited per site without leakage. The mice recovered well post operation.

CONCLUSIONS

The modified surgical procedures for transplantating iPSCs under the kidney capsule is easy, efficient, and results in significant reduction of cell leakage during transplantation.

T-2114

MULTI-ELEMENTAL ANALYSES REVEAL HIGH POTASSIUM AND ZINC LEVELS IN INDUCED PLURIPOTENT STEM CELLS DERIVED FROM SCHIZOPHRENIC PATIENT

Bruna Silveira Paulsen[1], Mariana Paranhos Stelling[1], Simone Coutinho Cardoso[2], Stevens Kastrup Rehen[1]

[A]Institute of Biomedical Sciences, [B]Institute of Physics, [1]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Schizophrenia has been defined as a neurodevelopmental disorder that affects approximately 1% of the world population. However, its causes have never been completely elucidated and most of the knowledge was acquired from post-mortem brain analyses or from non-neural cells. Human induced pluripotent stem cells (hiPSCs) are being considered extremely relevant as a complementary experimental model to study mechanistic insights of neurodevelopmental disorders and eventually to screen for new treatments. Hence, in this work we analyzed hiPSCs derived from 2 clones of a schizophrenic patient (SZP2 and SZP11) and controls (human embryonic stem cells and hiPSCs control) in order to unveil schizophrenia's impairments at the cellular and atomic level state through analysis of trace elements using the Synchrotron Radiation X-ray Fluorescence Spectroscopy.

Pluripotent colonies and neurospheres derived from hiPSCs from SZP and controls were irradiated with a spatial resolution of 20 µm to make elemental maps and quantitative chemical analyses. Multi-elemental analyses were performed and results did not revealed any significant difference amongst all pluripotent colonies. However, we observed high potassium (K) and zinc (Zn) levels on SZP-derived neurospheres compared to controls ($p<0.05$). These higher levels of K and Zn could be reverted to control levels by the treatment with the mood stabilizer, valproic acid.

These results contribute not only to a better understanding of the development of the disorder but also to highlight K and Zn as potential targets for diagnosis, treatment and drug screening.

T-2115

GENERATION OF INDUCED PLURIPOTENT STEM CELLS AND PRODUCTION OF LEUKEMIA INHIBITORY FACTOR (LIF) IN DOMESTIC CATS.

Ryoji Kanegi[1], Shingo Hatoya[1], Shigeo Takenaka[2], Toshiya Nishimura[1], Viscam Wijewardana[1], Kikuya Sugiura[1], Masahiro Takahashi[1], Noritoshi Kawate[1], Hiromichi Tamada[1], Toshio Inaba[1]

[A]Department of Advanced Pathobiology, Graduate School of Life and Environmental Sciences, [B]Department of Integrated Functional Biosciences, Graduate School of Life and Environmental Sciences, [1]Osaka Prefecture University, Osaka, Japan

We transduced four transcriptional factors (mouse Oct3/4, Sox2, Klf4, c-Myc) into fetal fibroblast cells of domestic cats with retrovirus vector. The cells were reseeded on SNL cells inactivated with mitomycin C and cultured in primate embryonic stem cell (ESC) medium supplemented with FBS or Knockout Serum Replacement (KSR). The Induced pluripotent stem-like (iPSC-like) shaped colonies appeared about ten days later after transduction. The colonies appeared more in the medium with KSR, however were maintained longer with FBS. We could establish an iPSC-like

cell line in the medium with FBS. The cell line was maintained about forty passages, expressed stem cell marker, possessed the ability to derivate into three germ layers, showed normal karyotype.
We cloned complete cDNA of leukemia inhibitory factor (LIF) from fetal fibroblast cells of domestic cats. The sequence revealed a homology of 81% or 92% of the amino-acid sequence of feline LIF with that of mouse or human. The feline LIF produced with pCold® TF DNA in *E.coli* was readily soluble and after purification showed bioactivity in maintaining the indifferent state of mouse embryonic stem cells and enhancing the proliferation of human erythrocyte leukemia cells. We therefore confirm the successful production for the first time of biologically active feline LIF within *E.coli*.

T-2116

JMJD3 NEGATIVELY REGULATES REPROGRAMMING BY PROMOTING INK4A/ARF EXPRESSION AND TARGETING PHF20 FOR UBIQUITINATION

WEI ZHAO, Qingtian Li, Helen Y. Wang, Rong-Fu Wang
The Center for Inflammation and Epigenetics, The Methodist Hospital Research Institute, Houston, TX, United States

Although somatic cell reprogramming can be achieved by several strategies, its efficiency and kinetics of iPSC generation are still very low, suggesting the existence of substantial genetic and epigenetic barriers during reprogramming. Global analysis of euchromatin dynamics during the reprogramming process has revealed orchestrated epigenetic changes at the histone modification level. Given the importance of epigenetic factors in defining cell lineages, it is reasonable to suggest that some of these factors are required for efficient somatic reprogramming, while others may function as negative regulators. Removal of such roadblocks to successful reprogramming will require increased insight into the molecular mechanisms by which epigenetic factors control cell lineage and hence the dynamic process of reprogramming. To define the facilitators and barriers of reprogramming, we identified Jmjd3 as a potent negative regulator of somatic cell reprogramming in screening studies using shRNA-mediated knockdown of a panel of histone-modifying proteins. Knockdown or ablation of Jmjd3 markedly enhanced the efficiency and kinetics of reprogramming, while ectopic expression of Jmjd3 inhibits this process. To determine the molecular mechanisms, we provide evidence that Jmjd3 partially inhibits iPSC reprogramming by promoting cell senescence through upregulation of p21 and Ink4a. However, the Jmjd3-deficient MEFs eventually underwent a senescence crisis after 5-7 passages, it is likely that transient effects of Jmjd3 deficiency on cell proliferation and senescence may have contributed to the improved efficiency and kinetics of reprogramming. Knockdown of Jmjd3, Ink4a/Arf or p21 alone by shRNAs increased reprogramming efficiency, compared to that in MEFs transduced with a control shRNA, but the efficiency nearly doubled with simultaneous knockdown of Jmjd3 and Ink4a/Arf or p21, suggesting that Jmjd3 might have additional effects on reprogramming. An extensive search for target molecules of Jmjd3 led to the identification of PHF20 (plant homeodomain finger protein 20). Jmjd3 targets PHF20 for ubiquitination and proteasomal degradation via the E3 ubiquintin ligase Trim26 in a demethylase activity-independent manner, suggesting that Jmjd3 and Trim26 control the protein level of PHF20 during reprogramming. Strikingly, PHF20 is required for maintenance and reprogramming and of iPSCS. Knockdown or ablation of PHF20 promotes ESC and iPSC differentiation, and blocks the reactivation of endogenous Oct4 and several ES marker gene expression, thus reducing reprogramming efficiency and leading to partially programmed cells. PHF20 binds to the Oct4 promoter region and interacts with Wdr5 and MOF complexes, while its ablation results in substantial reduction of Wdr5 and MOF binging to the Oct4 promoter. ChIP-Seq analysis further shows that many genes are co-bound by PHF20 and Wdr5. Our results implicate the Jmjd3-PHF20 axis as a key pathway in somatic cell reprogramming, and provide novel insights into the molecular mechanisms by which the Jmjd3-PHF20 axis controls this process.

T-2117

ANALYSES OF OXIDATIVE STRESS AND METABOLIC DYNAMICS IN PARK2 IPS CELLS-DERIVED NEURONS

Naoko Kuzumaki[1], Yoichi Imaizumi[1], Wado Akamatsu[1], Yohei Okada[1], Nana Izawa[2], Takuya Matsumoto[1], Takako Hishiki[3], Yoshiko Nagahata[3], Yukari Suda[4], Minoru Narita[4], Makoto Suematsu[3], Nobutaka Hattori[5], Hideyuki Okano[1]

[1]Keio University, School of Medicine, Department of Physiology, Tokyo, Japan, [2]Juntendo University Graduate School of Medicine, Department of Rehabilitation Medicine, Tokyo, Japan, [3]Keio University, School of Medicine, Department of Biochemistry, Tokyo, Japan, [4]Hoshi University, Department of Pharmacology, Tokyo, Japan, [5]Juntendo University Graduate School of Medicine, Department of Neurology, Tokyo, Japan

In the present study, we evaluated the change in the oxidative stress and energy metabolism of familial Parkinson's disease specific induced pluripotent stem cell (iPSCs)-derived neurons. iPSCs were generated from dermal fibroblasts isolated from two PARK2 patients carrying parkin mutations and two control subjects. All of the clones differentiated into neurons were including tyrosine hydroxylase-positive neurons. Neurons-derived from PARK2 iPSCs, but not fibroblasts or iPSCs, exhibited abnormal mitochondrial morphology and impaired mitochondrial homeostasis. Under these conditions, we examined oxidative metabolism in the iPSCs derived neurons by measuring the cellular levels of reduced glutathione (GSH). GSH reacts with reactive oxygen species (ROS) and is catalyzed by glutathione S-transferase. The levels of GSH in PARK2 iPSC-derived neurospheres were significantly lower as compared to that in control iPSC-derived neurospheres. We also examined ROS production using 2', 7'-dichlorodihydrofluorescin (DCF) fluorescence to measure the levels of intracellular oxidants. The DCF fluorescence intensity in the PARK2 iPSC-derived neurons was significantly higher than that in control iPSC-derived neurons. It has been reported that the Nrf2 pathway plays a cytoprotective role under conditions of ROS accumulation. The expression of Nrf2 pathway proteins, such as Nrf2 and NADH quinone oxidoreductase (NQO1), was significantly increased in PARK2 iPSCs-derived neurons, indicating an increased level of oxidative stress accompanied by activation of the Nrf2 pathway in PARK2 neurons. Whereas, Nrf2 has been shown to enhance several metabolic gene expressions in cancer cells. Following electrophoresis mass spectrometry (CE-MS) system, we found that parkin deficiency activated glycolysis and reduced mitochondrial respiration in neurons-derived from PARK2 iPSCs. These findings suggest that, although further studies are still needed, PARK2 iPSCs-derived neurons may affect oxidative stress and abnormal metabolism associated with the dysfunction of mitochondrial homeostasis.

T-2118

SORTING AND TRANSPLANTATION OF DOPAMINERGIC PROGENITOR CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Daisuke Doi, Asuka Morizane, Jun Takahashi
Dept of Clinical Application, Center for IPS Cell Res & Application, Kyoto city, Japan

Objective:
Cell replacement therapy using embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) could be applied for Parkinson's disease in the near future. In order to purify dopaminergic (DA) progenitor cells and exclude unwanted cells, we performed cell sorting using a floor plate marker, Corin. The DA characteristics of the sorted cells were analyzed in vitro and in vivo.
Methods:
We induced DA progenitor cells from human iPSCs (404C2, 836B3) and human ESCs (KhES-1) by dual SMAD inhibition and treatment with FGF8 and Shh. On day 12, we sorted Corin+ cells using a FACS (fluorescence-activated cell sorter). After an aggregation culture, we grafted the sorted cells into the striatum of the 6-OHDA lesioned rats. We evaluated behavior of the rats for 16 weeks, and then performed an immunohistological analysis of the grafts.
Results:
On day 12, a floor plate marker Corin was expressed by19.0±15.5% of total cells. The percentage of DA progenitor cells which express Foxa2 and Lmx1a was increased in the Corin+ cells compared to the unsorted cells; 75.5±8.3% vs. 47.4±6.6%, respectively.

The methamphetamine-induced rotation score was significantly improved both in the rats with unsorted and Corin+ cells. More TH+ neurons survived in the grafts derived from Corin+ cells, while the grafts of unsorted cells contained more proliferating cells and became larger in size.
Conclusion:
Sorting of DA progenitor cells using a floor plate marker, Corin, reduces a risk of graft overgrowth and leads to more condensed survival of DA neurons.

T-2122

DEVELOPMENT OF SAFE AND EFFICIENT CELL TRANSPLANTATION THERAPY FOR PARKINSON'S DISEASE USING NOVEL DIFFERENTIATION AND SORTING STRATEGY FOR HESC-, HIPSC- AND CYNOMOLGUS MACAQUE IPSC-DERIVED DA-NEURONS

Maria K. Sundberg, Tristan Lawson, Gaynor Smith, Arnar Astradsson, Helle Thomasen, Teresia Osborn, Oliver Cooper, Penelope Hallet, Ole Isacson
Neuroregeneration laboratory, Harvard Medical School, Belmont, MA, United States

The main pathophysiological symptoms of the Parkinson's disease (PD) are the loss of dopaminergic (DA) neurons in the ventral midbrain (VM). For the future treatment of Parkinson's disease with cell transplantation it is important to develop efficient differentiation methods for production of human iPSCs and hESCs-derived midbrain-type DA neurons. Here we describe an efficient differentiation and sorting strategy for gaining of DA-neurons from both human ES/iPS cells and non-human primate iPSCs. The use of non-human primate iPSCs for neuronal differentiation and autologous transplantation is important for pre-clinical evaluation of safety and efficacy of stem cell-derived DA neurons. The aim of this study was to examine the safety and efficacy of the sorted hESC-, hiPSC- and non-human primate-iPSC-derived DA neurons in PD-animal models; 6-OHDA lesioned rats and MPTP-treated cynomolgus macaques. According to our results NCAM+/CD29low sorting enriched most efficiently DA-neurons and these cells were also able to restore motor function of 6-OHDA rats after 16 weeks of transplantation. The transplanted sorted cells also integrated in the rodent brain tissue, with robust TH+ neurits outgrowth from the graft. After 1 year of autologous transplantation the primate iPSC-derived DA-neurons survived in primate striatum without any immunosuppression. This is important proof of concept for the feasibility and safety of iPSC-derived cell transplantation therapies for the future.

T-2123

MODELING PARKINSON'S DISEASE USING PATIENT IPSC-DERIVED DOPAMINERGIC NEURONS

Pengbo Zhang[1], Prachi Gujar[1], Ninuo Xia[1], Ha Nam Nguyen[1], Blake Byers[1], Patrick Lee[1], Theo Palmer[1], Birgitt Schuele[2], Renee Reijo Pera[1]
[1]Stanford Univ, Stanford, CA, United States, [2]The Parkinson's Institute and Clinical Center, Sunnyvale, CA, United States

Parkinson's disease (PD) is one of the most common neurodegenerative disorders in humans, due to the progressive loss of dopaminergic neurons in the midbrain. Currently, PD is incurable and methods that halt the progression of the disease are either ineffective or non-existent. Studies of PD in humans have been hampered by the lack of access to the affected neurons and a working model. Induced pluripotent stem cell (iPSC) technology has provided a gateway to study PD. Here we report the generation and characterization of disease specific iPSC lines. After extensive characterization, we have shown that these iPSC lines have a normal karyotype, express pluripotency markers and were able to differentiate into three germ layers both in vitro and in vivo. Furthermore, we could directly differentiate these cells into dopaminergic neurons and observe phenotypes such as α-Synuclein (α-Syn) abnormality and mitochondrial deficiency, which may be relevant to the disease etiology. Our findings suggest that these iPSC lines derived may be useful as a platform for investigative research in modeling human sporadic and genetic PD and understanding what molecules are involved in killing dopaminergic neurons.

T-2124

RECAPITULATING PARKINSON'S DISEASE **GENERATING PARKINSONIAN PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELL LINES, THEIR DIFFERENTIATION TO MIDBRAIN DOPAMINERGIC NEURONS AND THE SPECTRE OF RECOVERY**

Peter A. Barbuti[1], Jennifer L. Badger[1], Maebh Kelly[1], Lucy A. Crompton[1], Claire M. Rice[2], Alan L. Whone[2], James B. Uney[1], Maeve A. Caldwell[1]

[1]Dorothy Hodgkin Building, University of Bristol, Bristol, United Kingdom, [2]Department of Neurology, Frenchay Hospital, Bristol, United Kingdom

Objective
To derive patient-specific induced pluripotent stem cells (iPS) for use as a disease model in Parkinson's disease (PD).
Background
PD is primarily a neurodegenerative movement disorder clinically characterised by the loss of A9 dopaminergic neurons in the substantia nigra pars compacta of the midbrain. The disease is classically diagnosed by the motor symptoms of resting tremor, bradykinesia, rigidity and postural instability in addition to non-motor symptoms, such as depression. Not every PD sufferer presents with the same disease pathology thus making diagnoses and treatment difficult. There is estimated to be between 7-10 million people in the world with PD which per year costing an estimated $25billion per annum in the USA alone.
Methods
Lentiviruses encoding the pluripotency factors: Oct4, Sox2, Nanog, Lin28 and Klf4 were used to reprogram patient fibroblasts into iPS cells derived from a $2mm^2$ skin biopsy. An in-house 5-stage embryoid body (EB) based differentiation protocol was used to generate midbrain dopaminergic neurons. These neurons were then insulted with neurotoxins 6-hydroxydopamine (6-OHDA) and tert-butyl hydroperoxide (t-BHP); comparisons are then made between each derived iPS clone and also between Control, Idiopathic PD and Genetic PD patient-derived samples.
Results
It is possible to repeatedly and consistently generate iPS cells from PD patients with a variety of ages, symptoms, genetic and idiopathic pathologies. These iPS cells have then been characterised by the pluripotency markers: SSEA4, Tra-1-60 and Alkaline Phosphatase. Adapting an in-house protocol has enabled the generation of neurons characterised with a midbrain dopaminergic phenotype, notably for the markers of FoxA2, TH, Tuj1, Lmx1a, Nurr1 and Girk2. Data from insult experiments will also be presented.
Conclusions
It is possible to generate a disease-specific iPS cells from Parkinsonian patients. The generation of midbrain dopaminergic neurons enables PD to be modelled and *in-vitro*. This enables greater understanding on how and why this particular neuronal subset degenerates and what drugs/agents we can use to try and prevent/protect/regenerate them.

T-2125

INDUCED PLURIPOTENT STEM CELLS DERIVED FROM IDIOPATHIC PARKINSON'S DISEASE PATIENTS DIFFERENTIATE INTO MIDBRAIN DOPAMINERGIC NEURONS

Tetsuhiro Kikuchi, Asuka Morizane, Daisuke Doi, Keisuke Okita, Haruhisa Inoue, Jun Takahashi
Center for iPS Cell Research and Application, Kyoto Univ, Kyoto, Japan

Induced pluripotent stem cells (iPSCs) are promising source for cell replacement therapies. However, several problems remain to be solved before they can be used in clinical settings. Such problems include the use of animal-derived materials such as feeder cells, immune rejection by the hosts, and re-activation of virally transfected genes.
As for immune rejection, autologous transplantation of iPSCs derived from somatic cells of PD patients themselves can be a solution. Recent studies showed that iPSCs derived from patients with idiopathic Parkinson's disease (PD) could differentiate into dopaminergic (DA) neurons. Upon transplantation into PD model rats, they successfully re-

duced amphetamine- and apomorphine-induced rotational movements, indicating their contribution to functional recovery.
In this study, we show that iPSCs derived from patients with idiopathic PD can differentiate into dopaminergic neurons using our feeder-free culture method. First, we generated iPSCs from the dermal fibroblasts of 5 PD patients by reprogramming with episomal vectors. Generated iPSCs have similar morphology with human embryonic stem cells and expressed undifferentiated pluripotent marker genes such as Oct-3/4, Nanog, Tra-1-60 and SSEA-4. Polymerase chain reaction (PCR) confirmed that these iPSCs had no genomic integration of the episomal vectors. Then we differentiated these iPSCs into DA neurons. On the day 0 of neural induction, pluripotent iPSCs were seeded onto laminin E8 fragment-coated well plates in GMEM supplemented with 8% KSR. With the addition of Nodal and BMP inhibitors from day 0 to day 12, most of the cells were positive for PSA-NCAM, an early neuronal marker, at day 12. Purmorphamine and fibroblast growth factor 8 (FGF8) were added from day 1 to day 7 to induce DA neuronal progenitors. From day 12 onwards, the cells were replated with neurobasal medium supplemented with B-27 supplement, brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), dibutyryl cyclic AMP, and Ascorbic acid for further neuronal maturation. Immunohistochemistry and quantitative PCR indicated midbrain DA neuronal identity of the generated neurons. We compared the efficiency of DA neuronal differentiation, and found no significant difference between PD patient-derived iPSCs and normal control cells. We are now investigating the vulnerability of these induced DA neurons to oxidative reagent such as rotenone, 6-hydroxydopamine, and H2O2, and we found no significant difference so far.
In conclusion, we differentiated iPSCs derived from PD patients into midbrain DA neurons with our feeder-free differentiation method. We have no evidence indicating that idiopathic PD patient-derived iPSCs are more vulnerable than normal control cells, and these cells would be possible sources for cell transplantation therapy.

T-2126

EFFICIENT AND REPRODUCIBLE MYOGENIC DIFFERENTIATION FROM HUMAN IPS CELLS CAN RECREATE A PATHOLOGICAL CONDITION OF MIYOSHI MYOPATHY

Hidetoshi Sakurai[1], Akihito Tanaka[1], Katsuya Miyake[2], Knut Woltjen[1], Akitsu Hotta[1], Makoto Ikeya[1], Emi Shoji[1], Yasuko Manabe[3], Takuya Yamamoto[1], Takumi Era[4], Atsuko Sehara-Fujisawa[5], En Kimura[6]
[1]Ctr for IPS Cell Res & Application (CiRA), Kyoto, Japan, [2]Department of Histology and Cell Biology, School of Medicine, Kagawa University, Kagawa, Japan, [3]Department of Health Promotion Sciences, Graduate School of Human Health Sciences, Tokyo Metropolitan University, Hachioji, Japan, [4]Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto, Japan, [5]Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan, [6]Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan

**The establishment of human induced pluripotent stem cells (hiPSCs) has made possible patient-specific *in vitro* cell models of human disease. *In vitro* recreation of disease pathology from patient-derived hiPSCs depends on efficient differentiation protocols that lead to relevant adult cell types. However, myogenic differentiation of hiPSCs has faced obstacles, namely
low efficiency and/or poor reproducibility. Here we report the rapid, efficient
and reproducible differentiation of hiPSCs into mature myocytes. We demonstrate that inducible expression of *myogenic differentiation1* (*MyoD1*) in immature hiPSCs at least for 5 days drives cells along the myogenic lineage with efficiencies reaching 70%-90%. Myogenic differentiation driven by *MyoD1* is occurred even in immature hiPSCs near an undifferentiated status, without transitioning mesodermal differentiation. Myocytes induced in this manner reach maturity within two weeks differentiation, as assessed by marker gene expression and functional properties including *in vitro* and *in vivo* cell fusion and twitching in response to electric stimulation. Miyoshi Myopathy (MM) is a congenital distal myopathy caused by defective muscle membrane repair due to mutated Dysferlin. Using our induced differentiation technique, we successfully recreated the pathological condition of MM *in vitro*, demonstrating defective membrane repair in hiPSC-derived myotubes from a MM patient and phenotypic rescue by expression of full-length *Dysferlin*. These findings not only promote the pathologic-**

al investigation of MM, but also encourage further modeling of human muscular diseases using patient-derived hiPSCs.

T-2127

DEVELOPMENT OF AN IPSC BASED SCREENING PLATFORM FOR PARKINSON'S DISEASE DRUG DISCOVERY.

Cody Smith
The Scripps Research Institute and Cal Asia Pharmaceuticals, San Diego, CA, United States

Parkinson's disease (PD) is a debilitating neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra. Previously reported post-mortem analysis of patient-derived tissue from the substantia nigra has revealed cytotoxic aggregates of alpha synuclein associated with other proteins called Lewy bodies. A fully penetrant, aggressive form of familial PD is caused by the triplication of the gene encoding alpha synuclein, SNCA, which leads to a doubling in the expression levels of alpha synuclein and Lewy body formation. We plan to develop an in vitro model of PD through the dopaminergic differentiation of an SNCA triplication patient-derived iPSC line that was obtained from the Corriell Cell and DNA Repository. We have successfully cultured and biobanked this iPSC line for future drug screening applications. The cells were characterized using immunocytochemistry for pluripotency markers and embryoid body-based differentiation. Several iPSCs from an existing biobank of iPSCs generated from healthy donors were also characterized as controls.
We hypothesize that upon differentiation into dopaminergic neurons, the iPSCs with SNCA triplication will recapitulate the Parkinson's disease phenotype in vitro which will provide an ideal model for screening novel compounds that target the disease pathology. At CalAsia Pharmaceutics we are developing several small molecules for neurological disorders including molecules that inhibit FK506 binding proteins which are known to play a role in alpha synuclein aggregation, a hallmark of Parkinson's disease pathology. We are now beginning to selectively differentiate these iPSCs towards neural lineages that can be used for screening the FK506 inhibitor compounds. If this model is successful, it could potentially lead to in vitro validation of novel drugs for the treatment of Parkinson's disease.

T-2128

OXIDATIVE STRESS MEDIATES APOPTOSIS IN A HUMAN MODEL OF DANON DISEASE AND HEART FAILURE

Cynthia N. Perry[1], Sangyoon Han[1], Stacey D. Clegg[2], Kunfu Ouyang[1], Dekker Deacon[1], Ju Chen[1], Qiuming Gong[2], Zhengfeng Zhou[2], Neil C. Chi[1], Eric D. Adler[1]
[1]Medicine, UCSD, La Jolla, CA, United States, [2]Cardiovascular Medicine, OHSU, Portland, OR, United States

Autophagy plays a critical role in maintaining cell metabolism and aging. Furthermore, emerging evidence has highlighted the importance of autophagy in regulating cardiomyocyte bioenergetics, function and survival. However, the mechanisms responsible for cellular dysfunction and death in cardiomyocytes with impaired autophagic flux remain unclear. Danon disease is a familial cardiomyopathy associated with impaired autophagy due to mutations in LAMP-2. The objective of our study was to investigate the mechanisms involved in the development of heart disease due to the loss of LAMP-2. Using a human induced-pluripotent stem cell (iPSC) model of Danon Disease, we demonstrate that impaired autophagy increases mitochondrial oxidative stress and apoptosis due to accumulated autophagic vacuoles (AVs). Danon iPS cardiomyocytes recapitulated key features of the disease in vitro, including an increase in cell size, abnormal calcium handling and increased expression of brain natriuretic peptide. Treatment with the anti-oxidant N-acetylcysteine (NAC) significantly reduced oxidative stress and apoptotic cell death in Danon iPSC-cardiomyocytes. In summary, we used iPSC-derived cardiomyocytes to establish the relationship between impaired autophagy, mitochondrial oxidative stress and apoptosis in the pathogenesis of Danon disease. We also show that treatment with antioxidants decreases apoptosis, hence providing the basis for the development of therapeutics for autophagy-related human diseases such as heart failure, cancer and neurodegenerative disorders.

T-2131

NUCLEASE-INDUCED HOMOLOGOUS RECOMBINATION IN HIPSC DERIVED FROM PERIPHERAL BLOOD MONONUCLEAR CELLS OF PYRUVATE KINASE DEFICIENT PATIENTS

Zita Garate[1], Quintana-Bustamante Oscar[1], Laura Cerrato[1], Israel Orman[1], Fatima Rodriguez-Fornes[1], Tabita M. Maia[2], Leticia M. Ribeiro[2], Noemi Fusaki[3], Felix Garcia-Sanchez[4], Brian R. Davis[5], Agnes Gouble[6], Juan A. Bueren[1], Jose Carlos Segovia[1]

[1]Hematopoietic Innovative Therapies Division, CIEMAT, Madrid, Spain, [2]Serviço de Hematologia, Centro Hospitalar e Universitario de Coimbra, Coimbra, Portugal, [3]JST PRESTO and Ophthalmology, Keio University, Tokyo, Japan, Tokyo, Japan, [4]Histocompatibility and Molecular Biology Laboratory, Madrid Blood Transfusion Centre, Madrid, Spain, [5]Center for Stem cell and Regenerative Medicine, IMM-UTHSC, Houston, TX, United States, [6]Cellectis therapeutics, Paris, France

Induced pluripotent stem cells (hiPSC) obtained from patients with a diagnosed genetic disease, coupled with the targeted gene correction via homologous recombination (HR) are a promising alternative in the field of regenerative medicine. However, there are still methodological and safety aspects associated to these procedures, such as the need to avoid the persistence of the reprogramming genes, the generation of hiPSC clones from accessible tissues or the optimization of HR processes which need to be revised before considering its potential clinical use. Here, we used vectors based on RNA Sendai virus (SeV) to transfer and express the reprogramming genes. As a source of cells to reprogram we used peripheral blood mononuclear cells from patients with Pyruvate Kinase Deficiency (PKD), a genetic disease that causes non-spherocytic hemolytic anemia. Different hiPSC clones were obtained from two PKD patients and from a healthy donor with much higher efficiencies than those obtained using other reprogramming platforms. Pluripotency characteristics of the different hiPSC lines were tested by immunofluorescence, flow cytometry and RT-PCR arrays. Additionally, the disappearance of the reprogramming vectors was confirmed by RT-PCR after 15 passages in culture. Moreover, the hiPSC had a normal karyotype and no T or B cell receptor rearrangements. On the other hand, the genetic correction of PKD-iPSC was carried out by Meganuclease-mediated homologous recombination. We developed gene editing tools allowing insertion of a specific gene cDNA insert in intron 2 of the PKLR gene, facilitated by meganucleases. Experiments conducted in the erythroid line K562 have demonstrated that homologous recombination in the selected location was achieved. Experiments directed to generate disease free hiPSC by specific gene integration are now being conducted and results will be presented in the meeting.

T-2132

VASCULARIZED AND FUNCTIONAL HUMAN LIVER TISSUE FROM AN INDUCED PLURIPOTENT STEM CELL-DERIVED ORGAN BUD TRANSPLANT

Takanori Takebe, Keisuke Sekine, Masahiro Enomura, Hiroyuki Koike, Ran-Ran Zhang, Masaki Kimura, Takunori Ogaeri, Naoto Koike, Yasuharu Ueno, Yun-Wen Zheng, Hideki Taniguchi
Yokohama City University, Yokohama, Japan

Since the discovery of embryonic stem cells in1981, decades of laboratory studies have failed to generate a complex vascularized organ such as liver from pluripotent stem cells, giving rise to the prevailing belief that in vitro recapitulation of the complex interactions among cells and tissues during organogenesis is considered to be essentially impractical. One possible approach to create a complex and vascularized organ is to recapitulate the cellular interactions during organogenesis. Excellent animal studies revealed that the initial step of liver organogenesis, liver bud formation, requires the dynamic orchestrations between epithelial cells, mesenchymal cells and endothelial cells prior to vascular function. Here, we successfully created human liver bud-like tissues in vitro from human induced pluripotent stem cells (hiPSC) by recapitulating organogenesis. Without the aid of scaffolds, hepatic specified hiPSC were three-dimensionally organized into liver bud (hiPSC-LB) under the presence of endothelial and mesenchymal cells. Immunostaining and gene expression analyses revealed resemblance between in vitro grown hiPSC-LBs and in vivo liver buds. Human vasculatures in hiPSC-LB transplants became functional by connecting to the host ves-

sels within 48 hours. The formation of functional vasculatures stimulated the maturation of hiPSC-LBs into tissue resembling the adult liver. Highly metabolic hiPSC-derived tissue performed liver-specific functions such as protein production and human-specific drug metabolism without recipient liver replacement. Furthermore, transplantation of hiPSC-LBs onto mesentery rescued the drug-induced lethal liver failure model. To our knowledge, this is the first report demonstrating the generation of vascularized and functional human organ from pluripotent stem cells. Although efforts must ensue to translate these techniques to patients, our proof-of-concept, i.e. organ bud transplantation, provides a promising new paradigm of stem cell-based therapy beyond cell transplantation approach.

T-2133

NEW INSIGHTS INTO THE ROLE OF MIR-15A IN EARLY B CELL DEVELOPMENT IN A MOUSE MODEL OF CHRONIC LYMPHOCYTIC LEUKEMIA.

Chingiz Underbayev[1], Siddha Kasar[1], Yao Yuan[1], Heba Degheidy[2], Elizabeth Raveche[1]
[1]Pathology, UMDNJ, Newark, NJ, United States, [2]FDA, White Oak, MD, United States

New Zealand Black (NZB) mouse is a de-novo model of chronic lymphocytic leukemia (CLL) that has been studied as a model of B-cell lymphoproliferative disorder. This mouse exhibits a point mutation six bases downstream from pre-miR-16 region on chromosome 14, similar to the one seen in human CLL. In both NZB and CLL, the disease is characterized by the presence of a malignant clone of B-1 cells expressing CD5 and B220 and reduced expression of miR-15a/16. To date neither the cancer initiating population nor the chronic lymphocytic leukemia stem cell has been detected for this type of leukemia. To study early stages of B cell development in the context of CLL and shed light on the potential malignant cell origin of this disease, we generated induced pluripotent stem cells (iPS) from NZB spleen stromal fibroblasts. The pluripotency of NZB iPS cells was successfully confirmed by a number of tests including teratoma formation assay in NOD-SCID recipient mice. Our in vivo and in vitro studies on NZB iPS differentiation towards B-cell lineage cells revealed a substantial block in the maturation capacity of NZB iPS cells compared to wild type counterparts. Preliminary data suggests that exogenously delivered miR-15a/16-1 affects B-cell differentiation resulting in expression of higher levels of B220 (CD45R) and low to negative levels of CD5 surface marker, suggesting a loss of B-1 lineage cells. In addition, miR15a has been shown to be directly or indirectly involved in the regulation of IL7Ra expression during B cell maturation. Our results support the hypothesis that the NZB mouse model exhibits B1 lymphocyte skewing in the course of B cell development. This work will help further uncover new mechanisms of CLL development and shed light on the potential significance of miR-15a/16-1 as a B1 vs B2 lineage fate decision making factor.

T-2134

PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS PURGE MTDNA HETEROPLASMY WHILE MAINTAINING CARDIOGENIC CAPACITY

Ester Perales Clemente, Clifford D.L Folmes, Almudena Martinez-Fernandez, Xing Li, Amber McDonald, Devin Oglesbee, Sybil Hrstka, Carmen Perez-Terzic, Andre Terzic, Timothy J. Nelson
Mayo Clinic, Rochester, MN, United States

Mitochondrial defects cause a plethora of clinical manifestations, ranging from rare genetic disorders such as mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), to common diseases such as Alzheimer's disease, Parkinson's disease, cancer, cardiac disease or diabetes. Most of the ATP used by the cell is generated by mitochondria, but mitochondria have important roles in other cellular processes like adaptive thermogenesis, ion homeostasis, innate immune responses, production of reactive oxygen species (ROS), and programmed cell death. Mitochondria have a dual genetic control consisting of approximately 1500 genes distributed across the maternal mitochondrial DNA (mtDNA) and the Mendelian nuclear DNA (nDNA) that ensure optimal functionality of the oxidative phosphorylation system (OXPHOS). Therefore, mitochondrial disorders can be caused by mutations in both nuclear and mitochondrial genomes, and can display pathological phenotypes according to the mixture of mutant versus wild-type mtDNA, known as heteroplasmy.

Cytoplasmic hybrid cells or cybrids (produced by the fusion of a rho0 cell, without mtDNA, with a mitochondrial donor cytoplast), the gold standard in mitochondrial disease research, have confirmed the linkage for many disease-associated mtDNA mutations. However, cybrids have a fundamental limitation due to their innability to differentiate into tissue-specific progeny required for the study of cellular pathophysiology in mitochondrial diseases. Nuclear reprogramming of somatic tissues yields stem cells paired with differentiated tissues for genotype/phenotype analysis. Patient-specific induced pluripotent stem (iPS) cells offer a unique platform to bioengineer target tissues while maintaining the native mitochondrial and nuclear context.
We herein examined whether iPS cells derived from mitochondrial disease patient-specific somatic sources restructure mitochondrial heteroplasmy in clonal subpopulations. Patient-derived dermal fibroblasts with MELAS disease, demonstrated mitochondrial dysfunction with reduced oxidative reserve due to disease-causing heteroplasmy at position G13513A in the ND5 subunit of complex I. Bioengineered iPS cell lines acquired pluripotency with multi-lineage differentiation capacity, and consistently demonstrated reduced mitochondrial density and oxygen dependency compared to somatic sources. Sequence analysis in mtDNA of MELAS-iPS cells demonstrated a range of heteroplasmy (0% to 50%), establishing cell lines free of disease-causing mutations despite identical profiles within hypervariable regions of the mtDNA. Mitochondrial heteroplasmy within clonal iPS cell lines significantly decreased after 20-44 passages without jeopardizing pluripotency of the parental stem cell line. Thus, nuclear reprogramming of fibroblasts from a patient with mitochondrial disease produced isogenic pluripotent stem cell lines that could purge disease-causing mitochondrial genotypes. Furthermore, the recalibrated mitochondrial genotype was maintained throughout tissue-specific differentiation including cardiac lineages. Thus, heteroplasmic correction of patient-derived somatic cell lines enables comparison of genotype/phenotype relationships in stem cells and cardiac-specific progeny, providing a novel platform to probe cellular features of individual mitochondrial diseases.

T-2135

RETROVIRAL REPROGRAMMING OF HUMAN SKIN FIBROBLASTS TO PRODUCE INDUCED PLURIPOTENT STEM CELL LINES FOR THE GENERATION OF MOTOR NEURONS SPECIFIC TO ALS8

Jonathan T. Okubo[1], Helen C. Miranda[2], Alysson Muotri[2]

[1]California State University San Marcos, San Marcos, CA, United States, [2]Pediatrics, University of California San Diego, La Jolla, CA, United States

Induced pluripotent stem cells (iPSCs) have the unique ability to be differentiated into any human cell type. They also have the ability to self-renew infinitely. These two characteristics in combination give iPSCs immense promise in virtually all fields of medical research. Amyotrophic lateral sclerosis (ALS), or Lou Gehrig's disease, is a terminal neurodegenerative disease associated with the deterioration of motor neurons. ALS can be classified as familial or sporadic, with the sporadic form of the disease accounting for approximately 90-95% of ALS cases. ALS8 is a familial version of the disease caused by an autosomal dominant mutation in the vamp-associated protein B/C gene. Using human skin fibroblasts collected from two siblings from two separate families, one sibling presenting symptoms of ALS8, and one unaffected sibling, iPSC lines were generated by applying the Yamanaka factors Sox2, Oct4, c-Myc, and Klf4 via PEI retroviral transfection. Pluripotency was confirmed using immunofluorescent staining for pluripotency makers, karyotype analysis, as well as embryoid body formation and detection of the three germ layers. Future goals include generating motor neurons specific to ALS8, as well as a related, unaffected sample for comparison from the newly generated lines of iPSCs. These samples represent a viable model for many future studies, which may include further investigation of ALS8 on a molecular level, increased sufficiency in early diagnosis of the disease, as well as possible drug screening studies - all of which could potentially contribute to the development of a promising treatment.

T-2136

GENERATION OF INDUCED PLURIPOTENT STEM CELLS USING SKIN FIBROBLASTS FROM MYOCARDIAL INFARCTION PATIENTS IN FEEDER-FREE CONDITIONS

zhenyu lu, yushan zhang
Center for Reproductive Medicine, Tianjin Central Hospital of Obstetrics and Gynecology, Tianjin, China

Myocardial infarction (MI) is an increasing problem, but its pathogenesis remains unclear and more effective treatment strategies are required. Induced pluripotent stem cells (iPSCs) have recently been successfully generated using human somatic cells transfected with four transcription factors. In this study, we generated human iPSCs using human skin fibroblasts from MI patients, without the need for feeder-layer cells. These human MI-iPSCs expressed pluripotent genes and cell surface markers, and demonstrated normal proliferation. The iPSCs also showed in-vivo and in-vitro differentiation abilities, as indicated by teratoma and embryoid body formation, respectively. Moreover the iPSCs differentiated into cardiomyocytes and neuronal cells. In conclusion, human iPSCs were successfully generated from skin fibroblasts from MI patients in feeder-independent conditions, thus increasing their potential suitability for clinical applications. These results will further the study of MI pathogenesis and facilitate the safe downstream clinical applications of iPSC-based cell therapies.

T-2137

EFFICIENT PRODUCTION AND BANKING OF NEURAL PROGENITOR CELLS FROM INDUCED PLURIPOTENT STEM CELLS

Rita Martinez, Amber Neilson, Pat Blanner
Washington University in St. Louis, St. Louis, MO, United States

STEMCELL Technologies **STEMdiff™ Neural Induction Medium is an off the shelf product efficient at producing neural progenitor cells (NPCs). Our facility took a modified approach at producing, expanding and banking these NPCs that would maximize the number of cells produced /banked while reducing the overall cost of production. Several lines of induced pluripotent stems cells were used for this process. The vendor supplied protocol was modified to use non-tissue culture v-bottom 96-well plates and cell number per well was optimized to be between 45-65,000 cells/well. Results: The v-bottom plates allows for a lower cost and a neurosphere size which will produce a highly efficient number of rosettes (approx. 15+ per neurosphere). Once the rosettes are formed, we utilized** STEMdiff™ Neural Rosette Selection Reagent to collect rosettes in which 33% of them were banked and the remaining cells went into an adherent culture expansion. Cells were expanded to about 60-80% confluency then collected for banking and subsequent expansion. Approximately 20-30 million were collected for the first banking and additional 200,000 cells were used to expand for an additional bank of 10 million cells. Upon thawing a vial from each of these banks, we saw a 90-95% viability. In total, we were able to bank up to 40 million progenitors cells and retain 90-95% viability after banking these NPCs.

T-2138

GENERATION OF PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS INDUCED PLURIPOTENT STEM CELL LINES FOR UNDERSTANDING NEURODEGENERATION IN MULTIPLE SCLEROSIS

valentina fossati, Panagiotis Douvaras, Marco Calafiore
NYSCF, New York, NY, United States

Background: Multiple Sclerosis (MS) is the most common cause of neurological disabilities in young adults, affecting more than two million people worldwide. Although it is well known that during the course of MS immune cells attack the oligodendrocytes in the central nervous system, the initiating event that triggers the disease remains still elusive. Many progresses have been made over the last decade and several drugs, targeting the immune system, are now available for the majority of the patients affected by the relapsing-remitting form, which is characterized by recurrent and reversible episodes of focal inflammation. However, the majority of the RRMS patients will develop with time a secondary progressive (SPMS) form, distinguished by a steady irreversible neurological decline that is so far incurable. Furthermore, around 15% of patients are affected by a very severe primary progressive (PPMS)

form, characterized by a progressive course from onset and by an inescapable accumulation of neurological disabilities, despite any treatment.
As MS is exclusively a human disease, not spontaneously affecting other animals, one major drawback in the MS field is the lack of knowledge on human oligodendrocyte and neuronal biology, due to the limited availability of these cells for research.
Results: Here we show, for the first time to our knowledge, the successful generation of induced pluripotent stem (iPS) cells from PPMS patients and the further differentiation to oligodendrocytes and neuronal cell types.
Methods: This was accomplished by reprogramming fibroblasts from skin biopsies of PPMS patients using the mRNA/miRNA method. Moreover, we developed a new protocol for the differentiation of PPMS-iPS cells to immature oligodendrocytes. PPMS-derived iPS cells can successfully go through the major developmental stages of oligodendrocyte differentiation including neuroepithelium (Pax6+ cells) and glial progenitors (Olig2+ cells) and give rise to immature oligodendrocytes, expressing O4 surface antigen. The efficiency and the duration of our protocol are superior to the protocols previously published.
Conclusions: Axonal degeneration is now accepted as the principal cause of irreversible neurological disabilities in MS patients. PPMS-iPSC lines arise as a new powerful tool to generate unlimited numbers of human oligodendrocyte and neuronal populations to investigate the process of neurodegeneration. Unraveling the neurodegeneration mechanisms in MS is a critical step to identify novel therapeutic approaches that can arrest the escalating decline during the most severe secondary and primary progressive forms of the disease.

T-2141

REPROGRAMMING OF SOMATIC CELLS TO INDUCED-PLURIPOTENT STEM CELLS IN FEEDER-FREE, DEFINED, LOW-PROTEIN TESR™-E7™ MEDIUM

Wing Y. Chang, Arwen Hunter, Alvin Ng, Matthew Wong, **Erik Hadley**, Jennifer Antonchuk, Terry E. Thomas, Allen C. Eaves, Sharon A. Louis
Research and Development, STEMCELL Technologies, Vancouver, BC, Canada

The discovery
that nuclear reprogramming can be achieved by the exogenous expression of a
small number of transcription factors has opened up new possibilities in
regenerative medicine. The resulting pluripotent stem cells (PSCs), termed induced-pluripotent
stem cells (iPSCs), have been generated via exogenous expression of the
reprogramming transcription factors via multiple delivery systems and media
conditions, with a general movement towards more defined, clinically relevant
systems. Initially protocols achieved stable integration of the reprogramming
factor genes via retrovirus or lentivirus vectors. Protocols are now being developed for
non-integrating, transient expression systems such as episomal DNA vectors or
mRNA. In addition, defined and feeder-independent media conditions have been
developed to avoid the introduction of undefined factors in the iPSC derivation
step. Recently, Chen et al. (Nature Methods, 2011) published modifications of
the TeSR™ media formulation to create a simplified, low protein media for the feeder-free
maintenance of human PSCs (termed E8) and a medium for reprogramming of
fibroblasts into iPSCs (E7). Based on the
E8 formulation, we have recently released TeSR™-E8™ for maintenance of human PSCs,
and have now developed a simplified low-protein defined medium for
reprogramming: TeSR™-E7™. We
reprogrammed multiple fibroblast lines that were isolated from human adult and
neonatal tissue using both integrating and non-integrating vector systems, in
either TeSR™-E7™, TeSR™-E8™, or mTeSR1™. Feeder-free reprogramming of fibroblasts
in TeSR™-E7™ on Matrigel showed efficiencies of 0.125 ± 0.05 % (n=3) for human adult
fibroblasts, 0.0175% (n=2) for BJ fibroblasts, and 0.015% (n=1) for D551

fibroblasts. Thus, reprogramming efficiencies using TeSR™-E7™ are within the range of previously published reports for feeder-independent generation of iPSCs from somatic cells. When compared to our maintenance media TeSR™-E8™ and mTeSR1™, TeSR™-E7™ delivered a consistent 2-4 fold increase in the number of embryonic stem (ES) cell-like colonies. In addition, ES cell-like colonies were observed within 2 weeks after delivery of the reprogramming factors and were easily identified by live-cell staining with a Tra-1-60 antibody. By 3 to 4 weeks colonies were large enough to be picked and sub-cultured in feeder-free defined maintenance media. The putative iPSCs generated in TeSR™-E7™ were transferred to maintenance media TeSR™-E8™ or mTeSR™1, either on Matrigel or Vitronectin-XF coated plates, where they readily established thriving clonal cultures which could be further propagated as cell lines. The iPS cell clones derived in TeSR™-E7™ continued to exhibit human ES cell-like morphology and expressed pluripotent markers SSEA-4, SSEA-3, Tra-1-81, and Tra-1-60. Karyotypic analysis of cells from TeSR™-E7™ derived iPSCs clones did not show any gross chromosome anomalies. Further studies are ongoing to assess the pluripotency of iPSCs derived in TeSR™-E7™, including *in vitro* differentiation to 3 germ layers using STEMdiff™ kits for neural, definitive endoderm, and cardiomyocyte differentiation, as well as global transcriptome analyses to show that the clones have a fully reprogrammed transcriptome profile. Overall, TeSR™-E7™ offers a simplified, defined medium for feeder-free reprogramming of human fibroblasts to iPSCs, with efficiency of reprogramming equivalent to that in less defined systems.

T-2142

ANALYSIS OF IPS CELLS FROM FIBRODYSPLASIA OSSIFICANS PROGRESSIVA.

Makoto Hamasaki[1], Yoshinobu Hashizume[2], Tomohiko Katayama[1], Hirohiko Hohjoh[3], Noemi Fusaki[4], Yasuharu Nakashima[5], Hirokazu Furuya[6], Nobuhiko Haga[7], Takumi Era[1]

[1]Department of Cell Modulation, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto, Japan, [2]Program for Drug Discovery and Medical Technology Platforms, RIKEN, Wako, Japan, [3]National Center of Neurology and Psychiatry, National Institute of Neuroscience, Tokyo, Japan, [4]JST PRESTO and Ophthalmology, Keio University, Tokyo, Japan, [5]Department of Orthopaedic Surgery, Kyushu University School of Medicine, Fukuoka, Japan, [6]Department of Neurology, Neuro-Muscular Center, National Omuta Hospital, Omuta, Japan, [7]Rehabilitation Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Fibrodysplasia ossificans progressiva (FOP) is a rare congenital disorder characterized by progressive ossification in soft tissues. FOP is caused by mutations in activin receptor-like kinase 2 (ALK2) that cause its constitutive activation and result in dysregulation of bone morphogenetic protein (BMP) signaling.Here, we showed that generation of induced pluripotent stem (iPS) cells from FOP-derived skin fibroblasts is not successful. The treatment of ALK2 kinase inhibitor can restore the iPS cell generation, suggesting that abnormal BMP signaling can inhibit iPS cell generation. FOP-derived iPS cells express the pluripotent markers. However, they can not be maintained without ALK2 inhibitor. Gene expression analysis showed that mesodermal and endodermal markers were expressed in the iPS cells under the maintaining condition. To investigate this abnormal reprogramming, we monitored pluripotent surface marker such as TRA-1-60 during reprogramming. In early reprogramming stage, TRA-1-60 expression was decreased in FOP fibroblast. In addition, the expression of OCT4 and Nanog genes were reduced in TRA-1-60 positive FOP fibroblasts. These results suggest that FOP fibroblast reprogramming is incomplete. We searched for novel ALK2 kinase inhibitors using this system and found some new candidates for drug. This study demonstrates that the failure of

disease-derived iPS cell generation is useful for screening of novel therapeutic drugs and is the powerful tool to analyze reprogramming mechanism.

T-2143

A METHOD FOR EFFICIENT IPSC GENERATION USING NON-INTEGRATING PLASMID AND NFKB SIGNALING ACTIVATOR FROM HUMAN NEONATAL FIBROBLAST CELLS

Hyun Sub Hwang, Yong Joon Huh, Joo Hyun Park, Dong-Wook Kim
Department of physiology, Yonsei University, College of Medicine, Seoul, Korea, Republic of

Induced pluripotent stem cells (iPSCs) can be generated from various types of somatic cells by reprogramming factors identified by Yamanaka group (Oct4, Sox2, Klf4, and Myc). The efficiency of iPSC generation is known to be affected by the age of donor, the types of cell used, the passage number, and the employed reprogramming methods. In order to minimize variable conditions of reprogramming, we utilized an improved plasmid endcoing 5 reprogramming factors as a single polycistronic unit (Oct4, Sox2, Klf4, c-Myc and Lin28) and human neonatal fibroblast cells. The non-integrating plasmid enabled iPSC colonies to be formed within 2 weeks after a single transfection by electroporation. The efficiency of deriving of iPSCs from human neonatal fibroblast through our method was approximately 1%. To further enhance the efficiency of nuclear reprogramming, TLR-NF-κb signaling was adapted. Here, we found that the nuclear reprogramming efficiency was greatly enhanced by treatment of the NF-κb signaling activators, and this was decreased in IκB mutant implying the role of NF-κb signal played during reprogramming. In this study, we report identification of the key limiting factors that constrain the efficiency of reprogramming.
This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) of MEST (2012M3A9B4028631, 2012M3A9C7050126), as well as by a grant from the Korea Health technology R&D Project of ME (A120254), Republic of Korea.

T-2144

IN VITRO MODELLING OF ARRYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY (ARVC) USING INDUCED PLURIPOTENT STEM CELLS

Azra Fatima[1], Guoxing Xu[1], Martin Lehmann[1], Andreas Broedehl[2], Sven Dittmann[3], Matthias Linke[4], Kaifeng Shao[1], Ulrich Zechner[4], Hans-Christian Hennies[5], Martin Farr[3], Jürgen Hescheler[1], Hendrik Milting[3], Tomo Šarić[1]
[1]University of Cologne, Institute for Neurophysiology, Cologne, Germany, [2]University Hospital of the Ruhr-University of Bochum, 2Erich and Hanna Klessmann-Institute for Cardiovascular Research and Heart and Diabetes Center, Bochum, Germany, [3]University Hospital of the Ruhr-University of Bochum, Erich and Hanna Klessmann-Institute for Cardiovascular Research and Heart and Diabetes Center, Bochum, Germany, [4]Johannes Gutenberg University, Institute for Human Genetics, Mainz, Germany, [5]University of Cologne, Cologne Center for Genomics, Clinic III for Internal Medicine, Cologne, Germany

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited heart muscle disease, frequently accompanied by sudden cardiac death and terminal heart failure. Molecular genetic screening for mutations in the desmosomal candidate genes JUP, DSG2, DSC2, DSP and PKP2 known to be associated with ARVC revealed a novel desmin-mutation p.N116S which is located in segment 1A of the desmin rod domain. The mutation affects filament formation leading to protein aggresomes in cardiac and skeletal muscle without signs of an overt clinical myopathy in vivo. We have generated iPS cells from a patient carrying a novel spontaneous heterozygous autosomal dominant mutation in the gene desmin (N116S) in order to elaborate on the role of this point mutation in the development of ARVC.
The ARVC- iPS cells were indistinguishable from human embryonic stem cells and showed typical features of pluripotent cells. Spontaneously beating cardiomyocytes (CM) were readily obtained by in vitro differentiation of ARVC-iPS cells. Electrophysiological properties of ARVC-CM were studied using multielectrode arrays and patch clamp. Although the ARVC-CM exhibited higher beating frequency, there was no significant difference in action potential

parameters and response to ß-adrenergic and muscarinic stimulation to control cells. The expression of desmin at the transcript level was similar in ARVC-CM and control CM. Immunocytochemical assessment of desmin expression and intracellular distribution in ARVC-CM did not show the expected desmin aggregate formation even after 90 days of CM maturation. This could be attributed to the heterozygosity in the mutant desmin gene expression. The control iPS-CM contained filamentous desmin but the ARVC-CM showed an intermediate pattern lacking a highly organized filamentous form of desmin. In our experiments the ARVC-specific cardiomyocytes so generated in vitro do no recapitulate the patient's disease phenotype due to lack of aggresomes in the cardiac cells. Further analyses are being carried out to assess the functional and structural consequences of mutant desmin expression in CM.In vitro and in vivo maturation of these cardiomyocytes will be helpful to assess the functional impact of the mutant desmin at the organ level. As such, ARVC-CM serve as an important platform to study the pathophysiological mechanism of this complex cardiac disorder.

T-2145

KERATINOCYTE DERIVED IPS CELLS FROM 22Q13.3 DELETION SYNDROME PATIENTS

Stefan Liebau, Leonhard Linta, Tobias M. Boeckers
Institute for Anatomy & Cell Biology, Ulm University, Ulm, Germany

Plucked human hair have already been shown to be a valuable source for keratinocytes which can be efficiently reprogrammed to hiPS (human induced pluripotent stem) cells. Keratinocytes have a much higher reprogramming efficiency compared to fibroblasts or other cell types. Our group has successfully used this method to collect samples from several 22q13.3 deletion syndrome (also known as Phelan-McDermid Syndrome) patients. After initial outgrowth, the keratinocytes were reprogrammed to hiPS cells via lentiviral infection using REFs (rat embryonic fibroblasts) as feeder cells. We could show in an earlier study that REFs improve the reprogramming efficiency and success rate of obtaining hiPS clones. To generate virus free stem cell lines the loxp site containing reprogramming cassette was excised by addition of recombinant Cre protein to the cells. The arising clones were negative for the reprograming cassette, showed all pluripotency hallmarks and were confirmed to carry the deletion on chromosome 22. These cells can be used for neural differentiation to study the neurogenesis and synaptogenesis of diseased cells in vitro. Comparison of their phenotype with healthy control cells are utilized to study the disease mechanism in detail.

T-2146

ESTABLISHING IN VITRO MODELS OF AUTISM SPECTRUM DISORDERS USING INDUCED PLURIPOTENT STEM CELLS

Katya Mc Donagh[1], Philip H. Schwartz[2], David J. Brick[3], Timothy O'Brien[1], Frank Barry[1], Sanbing Shen[1]
[1]School of Medicine, National University of Ireland Galway, Regenerative Medicine Institute, Galway, Ireland, [2]Children's Hospital of Orange County Research Institute, Center for Translational Research, California, CA, United States, [3]Center for Translational Research, Children's Hospital of Orange County Research Institute, California, CA, United States

Autism, which has been estimated to affect 1 in 88 children, a number that has climbed steadily in the recent decades, is not believed to be a single disorder but rather a whole spectrum of disorders (ASD) with varying severity and multiple genetic risk factors. Autism is characterized by developmental delay, impaired social communication and stereotyped behaviors. Symptoms are often recognized in the first two years of a child's development. Drug therapies are available which can help improve the quality of life including better management of seizures, depression, hyperactivity and anxiety. Early interventions such as speech, language and behavioural therapy have been shown to reduce severity of stereotyped and repetitive behaviors and improve upon social skills in the ASD patient. Current therapies only alleviate some of the the patient symptoms to an extent. Induced pluripotent stem cells (iPS cells) have been developed to establish in vitro models of neuropsychiatric disorders, e.g. schizophrenia. Such models provide the opportunity to investigate synaptic development and function of a specific disorder. We have reprogrammed patient fibroblasts containing mutations in the FMR1 gene and a deletion in the NRXN1 gene us-

ing a single lentivirus cassette containing the four factors, OCT4, SOX2, KLF4 and c-MYC. iPS clones with the mutation/ deletion along with non-affected control iPS clones have been identified and isolated for expansion. We are currently characterizing each cell line to confirm their pluripotency and partially knock down the NRXN1 gene in non-affected iPS lines prior to differentiating the FXS/ NRXN1 iPS lines to neurons. We are investigating neuronal development throughout iPS cell differentiation and comparing gene expression and synaptic formation and function in the FXS/ NRXN1 derived lines during neuronal differentiation.

T-2148

IMPAIRED AUTOPHAGY IN A LIPID STORAGE DISORDER NIEMANN-PICK TYPE C1 DISEASE WHERE UPREGULATION OF AUTOPHAGY IS A POSSIBLE THERAPEUTIC STRATEGY

Sovan Sarkar, Dorothea Maetzel, Rudolf Jaenisch
Whitehead Institute for Biomedical Research, Cambridge, MA, United States

Autophagy dysfunction has been implicated in the accumulation of misfolded proteins and in cellular toxicity in several diseases. Whether defects in autophagy also contribute to the pathology of lipid storage disorders is not clear. Here we show defective autophagy in Niemann-Pick type C1 (NPC1) disease associated with cholesterol accumulation, where maturation of autophagosomes is impaired due to defective amphisome formation because of disturbed autophagic traffic and failure to recruit vesicle fusion proteins. Proteomics analysis linked NPC1 deficiency to aberrant intracellular trafficking and to defective mitophagy, whereas endocytic traffic and lysosomal proteolysis were not overtly affected. Our data suggest a role of the NPC1 protein in mediating autophagic flux. Compromised autophagy was shown in the liver and cerebellum of Npc1 mutant mice, and in hepatocyte-like cells derived from NPC1 patient-specific induced pluripotent stem cells (iPSCs). Of potential therapeutic relevance is that exposure of mutant cells to HP-β-cyclodextrin, which is being used to lower cholesterol levels in animal models and currently tested in patients, has an undesirable side-effect in impeding autophagy, whereas upregulation of autophagy rescues its defects in NPC1 mutant fibroblasts and patient-specific iPSCs, and thus could be a possible treatment strategy for lipid/lysosomal storage disorders. Our data suggest that a partial depletion of cholesterol in NPC1 mutant cells with a lower dose of HP-β-cyclodextrin, such that autophagic flux is not further perturbed, coupled with an autophagy inducer, could provide a rational combination treatment for NPC1 disease.

T-2151

IDENTIFICATION OF BIOMARKERS FOR HEREDITARY CARTILAGE DISEASE USING CHONDROCYTES DERIVED FROM PATIENTS-SPECIFIC IPSCS

Makoto Watanabe[1], Koji Yokoyama[2], Makoto Ikeya[3], Takayuki Tanaka[4], Ryuta Nishikomori[3], Megumu Saito[3], Katsutsugu Umeda[3], Toshio Heike[3], Taka-Aki Sato[5], Junya Toguchida[6]
[1]Life Science Research Center, Technology Research Laboratory / Department of Tissue Regeneration, Shimadzu Corporation / Institute for Frontier Medical Science, Kyoto University, Kyoto, Japan, [2]Department of Cell Growth and Differentiation / Department of Pediatrics, Center for iPS Cell Research and Application / Gradate School of Medicine, Kyoto University, Kyoto, Japan, [A]Department of Cell Growth and Differentiation, [B]Department of Clinical Application, [3]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [4]Life Science Research Center, Technology Research Laboratory, Shimadzu Corporation, Kyoto, Japan, [5]Department of Tissue Regeneration / Department of Cell Growth and Differentiation, Institute for Frontier Medical Sciences / Center for iPS Cell Research and Application / Gradate School of Medicine, Kyoto University, Kyoto, Japan

Chronic infantile neurological cutaneous articular (CINCA) syndrome is an autoinflammatory disease, characterized by uriticarial rash, central nervous system disorders including meningitis and deafness, and articular symptom. Nod-like receptor pyrin domain-containing 3 (NLRP3) is identified as a responsible gene for CINCA syndrome. NLRP3 forms inflammasomes and regulates the release of proinflammatory cytokines such as interleukin-1β (IL-1β) in response to extrinsic pathogens and intrinsic danger signals. In CINCA patients, secretion of mature IL-1β is de-regu-

lated and accelerated because of constitutive active mutants of NLRP3. The NLRP3 gene is expressed in chondrocytes, and the arthropathy of patients is the deformity of epi-metaphases of long bones with abnormal proliferation of calcified chondrocytes. These suggest the involvement of NLRP3 in growth and differentiation of growth plate chondrocytes. To elucidate molecular mechanisms of arthropathy, we generated induced pluripotent stem cells (iPSCs) with and without mutant NLRP3 from CINCA patients with mosaicism. Chondrogenic progenitors were induced from each type of iPSCs, from which proteins were extracted and subjected to proteome analysis using two-dimensional electrophoresis. Protein spots with altered expression levels (>1.5-fold) between mutant and wild type were subjected to peptide mass fingerprinting or MS/MS analyses with tandem mass spectrometry for protein identification. To gain further insights, the proteins with altered expression levels were then subjected to pathway analysis using Pathway Studio 9.0 to identify significantly associated biofunctions. The findings in the present study may provide a useful marker for elucidating the disease pathogenesis and for developing treatment for arthropathy.

T-2152

REPROGRAMING OF COMMON MARMOSET SOMATIC CELLS BY INTEGRATION-FREE METHOD USING EPISOMAL VECTOR

Shin-ichi Inoue[1], Hiroshi Igarashi[1], Hideyuki Okano[2], Erika Sasaki[1]

[1]Department of Applied Developmental Biology, Central Institute for Experimental Animals, Kawasaki, Japan, [2]Department of Physiology, Keio University, School of Medicine, Tokyo, Japan

Common marmoset *(Callithrix jacchus),* one of the New World monkey, is small body size, easy to handle and similar to human metabolic pathway, physiological and anatomical characteristics. Furthermore, marmoset is prolific animal and transgenic non-human primate models for human disease are available only in this species. Pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are expected to use for stem cell based regenerative medicine including auto/xeno graft. Recently, integration-free method using adeno virus, sendai virus and episomal plasmid vector have been developed and these vectors are thought to be low risk of oncogenesis. Therefore, for clinical use, iPSCs generated using integration-free method are considered to be applied to stem cell therapy. To evaluate safety and efficacy of the regenerative medicine using the pluripotent stem cells, non-human primate models are essential. Previously, we have generated marmoset iPSCs from liver cells using retrovirus vector to establish preclinical study system for regenerative medicine. However, to evaluate safety and efficacy of the stem therapy, our iPSCs are not suitable models because transgenes integrated into the genome. To assess safety and efficacy of the stem cell therapy with accuracy, integration-free iPSCs must be used in the preclinical study. However, integration-free iPSCs has not yet generated on marmoset somatic cells. In this study, we perform the reprogramming of marmoset somatic cells by integration-free method using episomal vector. Marmoset fetus fibroblasts were inducted with episomal vectors encoding inducible factors Oct3/4, Sox2, Klf4, L-myc, Lin28 and shp53. On day7, the induced cells were replaced onto feeder layer with ES medium. After 20 days of induction, several colonies resembling marmoset ESCs appeared, and these colonies showed alkaline phosphatase activity. Transcription of endogenous Oct3/4, Klf4, Sox2 and Nanog mRNA were confirmed in these colonies by reverse-transcription polymerase chain reaction. Moreover, expression of SSEA-3 and SSEA-4 were confirmed on these colonies by immune stain, although SSEA-1 expression was not observed. In this culture condition, undifferentiated states remain over 30 passages. These results suggest that marmoset fetus fibroblast cells are able to reprogram to undifferentiated state by episomal vector, and it can be used as non-human primate models for regenerative medicine.

T-2153

MODELING CONGENITAL DISORDERS OF GLYCOSYLATION IA VIA GENERATION OF INDUCED PLURIPOTENT STEM CELLS (IPSCS) FROM CDG IA PATIENT´S FIBROBLASTS

Christina T. Müller[1], Dirk Hoffmann[2], Doris Steinemann[3], Axel Schambach[2], Falk F. Buettner[1]

[A]Institute for Cellular Chemistry, [B]Institute for Experimental Hematology, [C]Institute of Cell and Molecular Pathology, [1]Hannover Medical School, Hannover, Germany

Congenital disorders of glycosylation (CDGs) are genetic diseases mainly affecting N-glycan synthesis . N-glycosylation has big influence on correct protein folding and stability, implicating great importance for both inter- and intracellular signaling. A total loss of N-glycans is lethal. Thus, type I CDG mutations display hypomorphic alleles, not complete knockouts, which are generally associated with reduction of the respective enzyme activity. All type I CDGs lead to hypoglycosylated proteins. Mutations in the phosphomannomutase 2 (PMM2) locus causes CDG Ia, the most common form of CDGs, with over 600 cases worldwide. Clinical symptoms are broad and involve many organ systems especially the brain, liver, immune system and heart leading to psychomotor retardation and cardiomyopathy, for example. Currently, no treatment options exist.
To study the impact of reduced PMM2 activity in early development, induced pluripotent stem cells (iPSCs) from patients fibroblasts suffering from a mutation in the PMM2 locus were created by reprogramming applying lentiviral gene transfer of the four transcription factors OCT-3/4, SOX2, KLF4 and c-MYC.
The generated CDG Ia iPSCs showed no morphological differences compared to wild type iPSCs. Real-time PCR analysis and fluorescence microscopy revealed similar expression levels of the pluripotency markers OCT-3/4, NANOG and SSEA-4 compared to healthy controls. Significant chromosomal abnormalities of the generated iPSCs were excluded after comparative genomic hybridization analysis (Array CGH). Vector copy number analysis revealed only two integrations sites suggesting minimal perturbances of gene expression profile.
Initial lectin-blots of whole cell lysates of CDG Ia-iPSCs showed hypoglycosylation compared to wild-type iPSCs and human embryonic stem cells implicating a disease manifestation already on the embryonic level.
This model will create an important platform to dissect the effects of glycosylation in pluripotent stem cells and development.

T-2154

GENERATION OF RAPIDLY-DIFFERENTIATING INDUCED PLURIPOTENT STEM CELLS

Koji Nishihara, Ryo Yamaguchi, Wado Akamatsu, Hideyuki Okano
Physiology, Keio University School of Medicine, Tokyo, Japan

Pluripotent stem cells (ES/iPS cells) have ability to differentiate into various types of somatic cells and are expected to be used in the future regenerative medicine. However, neural stem cells, which are derived from ES/iPS cells, differentiate into their progeny apparently slowly compared to neural stem cells taken from embryos. These features of ES/iPS-derived neural stem cells force to perform a long-term cultivation to obtain mature neural cells and might induce residual differentiation-resistant cells that induce tumors in host animals. Therefore, it is very useful for regenerative medicine and iPS researches, if we could establish iPS clones that induce rapidly differentiating neural stem cells.
By modifying reprogramming process, we tried to make fast-differentiating iPS clones from mouse embryonic fibroblasts. While primary neural stem cells derived from iPS cells established by the previously reported conventional method (Takahashi et al., Cell, 2006) are mostly neurogenic, neural stem cells derived from iPS cells established by our modified protocol are highly gliogenic even at early passages. During the recurrent passages, these gliogenic neural stem cells developed into mature neural stem cells significantly faster than neural stem cells derived from control iPS cells that were established by the conventional method. Remarkably, these two groups of iPS cells (‘fast-differentiating’ or ‘conventional’ iPSCs) were derived from genetically identical fibroblasts, and every stage of cultural condition and transfection procedure was similar between the two with the exception of the culture condition in the reprogramming stage. Interestingly, these fast-differentiating iPS cells are not significantly different from ‘conventional’ iPS cells in the morphology and the expression of pluripotent and neural genes analyzed by microarray analysis. However, they also revealed significant differences between these two groups in the expression of several specific genes that have never known in connection with neural differentiation or reprogramming. Some of these gene expression patterns, including several surface markers, were also confirmed by quantitative real-time PCR.

In conclusion, we succeeded in making that have distinct properties from 'conventional' iPS cells even they were generated from the same parental cells, and some genes that expressed uniquely in fast-differentiating iPS cells were detected. These genes might be associated with a critical determinant of the differentiation potential of ES/iPS cells. Though the resistance of ES/iPS cells to differentiation into aimed cells is still a big barrier to promoting regenerative medicine at present, we could sort out 'good 'ES/iPS clones, which are genuinely competent for the regenerative medicine by applying our findings.

T-2155

GENERATION OF ENDOTHELIAL CELLS FROM SCALABLE CULTURES OF UNDIFFERENTIATED HUMAN PLURIPOTENT STEM CELLS

Ruth Olmer[1,3,4], Sven Becker[5], Robert Voswinckel[5], Ulrich Martin[2]
[A]REBIRTH Cluster of Excellence, [B]LEBAO, [1]Hannover Medical School, Hannover, Germany, [2]BREATH Biomedical Research in Endstage and Obstructive Lung Disease, Hanover, Germany, [3]Member of German Center for Lung Research, Hannover, Germany, [4]Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany

Full endothelialisation of gas exchange membranes in extracorporal membrane oxygenation (ECMO) devices for improved hematocompatibility, or cell therapy of pulmonary hypertrophy requires large amounts of (patient-specific) endothelial cells (ECs). The isolation of ECs from peripheral blood or explanted vessels is well established however especially cells from older individuals show a limited proliferation capacity. Patient specific ECs from pluripotent stem cells (hiPSCs) might be an alternative suitable cell source. The opportunity to generate large amounts of undifferentiated hiPSC in defined media and under scalable monitored conditions allows for the generation of cell numbers in dimensions which are suitable for cellular therapies. By differentiation of these well monitored cell populations a virtually unlimited number of autologous ECs may become available for disease modelling and biofunctionalization of ECMO devices.
Utilization of BMP4 and VEGFA for the differentiation of the scalable suspension cultures resulted in up to 12% of CD31 positive cells. With substitution of BMP4 by a small molecule GSK3ß inhibitor the amount could be increased to 20% of CD31 positive cells on day 10 of differentiation.
FACS-sorted $CD31^+$ iPSC derivatives will be characterized in detail with respect to their molecular phenotype, proliferative capacity and functionality. In addition, the generation of transgenic hiPSC reporter lines, which express a fluorescence reporter / antibiotic resistance under the control of EC specific promotors (VE cadherin or CD31) for further improvement of differentiation is in progress. Resulting patient- (and lung disease-) specific iPSC-derived ECs will represent a novel cell source for disease modelling or biofunctionalization of gas exchange membranes. In addition, TALEN-based gene correction in iPSCs might enable novel concepts of *ex vivo* gene therapy for respiratory diseases.

T-2156

ACCELERATED NEURAL DIFFERENTIATION AND LOSS OF SELF-RENEWAL IN SMITH LEMLI OPITZ SYNDROME PATIENT IPS CELLS

Kevin Francis, Christopher Wassif, Amy Ton, Heiner Westphal, Forbes D. Porter
NICHD-NIH, Bethesda, MD, United States

Smith-Lemli-Opitz Syndrome (SLOS) is an autosomal recessive disorder caused by mutations in the gene encoding 7-dehydrocholesterol reductase (DHCR7). DHCR7 mutation reduces cholesterol synthesis, causing accumulation of cholesterol precursors 7- and 8- dehydrocholesterol (DHCs) and cellular toxicity. Patient outcomes range from neonatal lethality to cognitive dysfunction. Classical SLOS patients exhibit microcephaly, dysplasia of the cerebellum and absence of the corpus callosum, resulting in motor dysfunction, cognitive disabilities and autistic behavior. While dietary cholesterol may treat SLOS in peripheral tissues, it fails to cross the blood-brain-barrier to treat the neurological aspect of SLOS. As animal models are limited by early lethality, we have developed induced pluripotent stem cells (iPSCs) from patients diagnosed with classical or severe SLOS to study disease development and identify new therapeutics. Comparison of SLOS iPSCs to ES and iPSC controls via chromosomal, molecular and cellular assays revealed no differences in pluripotency when cultured with exogenous cholesterol. A biochemical hallmark of SLOS is the accumulation of DHCs and reduced cellular cholesterol in the absence of exogenous cholesterol. To examine how SLOS iPSCs respond to cholesterol free conditions, cells were transferred to a defined, cholesterol free pluripotent media. While controls never accumulate DHCs, GC-MS analysis of SLOS iPSCs cultured under cholesterol free conditions revealed a time-dependent increase in DHCs and decreased cholesterol corresponding to SLOS severity. Severe SLOS iPSCs revealed a nearly complete loss of DHCR7 activity while classical SLOS iPSCs exhibited more moderate changes. While controls remained pluripotent, SLOS iPSCs, amazingly, exhibited spontaneous differentiation, predominately to neural progeny. Expression of neural markers and loss of pluripotency was confirmed by immunocytochemistry, FACS, and qPCR. Further, when subjected to neural differentiation assays, SLOS iPSCs displayed disorganized columnar progenitor formation and accelerated neuronal development. A whole genome array time-course comparison of control and SLOS iPSCs cultured in the absence of cholesterol revealed the upregulation of neural gene expression subsequent to loss of key self-renewal signaling molecules and activation of key transcription factors. Rescue of this differentiation could only be achieved through combinatorial supplementation of cholesterol and small molecules. Current studies are further analyzing identified signaling pathways and examining neural populations in an SLOS mouse model. These are the first data to show the effects of DHCR7 mutation on human neural differentiation and suggest stem cell defects may underlie the functional and developmental issues associated with SLOS.

T-2158

COMPARISON OF MIRNA AND MRNA TRANSCRIPTION PATTERNS IN HEPATOCYTES DERIVED FROM HESC AND HIPSC

Jane Marie Synnergren[1], Josefina Edsbagge[2], Marie Rehnström[2], Mikael C. Englund[2], Frédéric Lemaigre[3], Petter Björquist[2]
[1]University of Skövde / Cellectis, Skövde, Sweden, [2]Cellectis, Gothenburg, Sweden, [3]Université catholique de Louvain, Brussels, Belgium

There is a huge need for an unlimited supply of functional human hepatocytes relevant for cell replacement therapies, drug discovery, and toxicology testing. Today human primary hepatocytes (hPH) and various animal models are used, but these model systems are marred by several disadvantages. An *in vitro* model system, based on human stem cells, has great advantages and provides a stable and unlimited supply of human hepatocytes. Efficient methods for differentiation of hepatocytes from human embryonic stem cells (hESC), and recently, also from human induced pluripotent stem cells (hiPSC) are now available and these cells can provide a defined and renewable source of human hepatocytes. The *in vitro* derived hepatocytes highly resemble cells in the *in vivo* organ, although not fully functional in all hepatic aspects. To identify and understand these functional differences, more characterization is still required of human pluripotent stem cell (hPSC)-derived hepatocytes.
Present study investigates the transcriptional differences between hESC-derived and hiPSC-derived hepatocytes, including both microRNA (miRNA) and mRNA expression. Samples were harvested at defined time points during the stepwise differentiation process, and fetal and adult liver samples were encompassed as controls. Genome wide

microarrays were applied for the global transcriptional profiling of miRNAs and mRNAs, and differentially expressed transcripts during the hepatic differentiation of hESC and hiPSC were identified. Comparisons of *in vitro* derived hepatocytes with the control samples were also performed, with the purpose to identify genes with association to the incomplete hepatic functionality observed in hPSC-derived hepatocytes. We also performed pathway analysis using the KEGG pathway database, to identify pathways, which deviates between hESC- and hiPSC-derivatives as well as between *in vitro* derived hepatocytes and *in vivo* samples. Protein interaction networks were generated from differentially expressed genes to identify hub-proteins with key functionality during hepatic differentiation.
In this study transcriptional signatures for both hESC- and hiPSC-derived hepatocytes have been identified, including both mRNA and miRNA molecules. Generally, when comparing the hESC- and the hiPSC-derivatives, a high level of similarity was demonstrated, but also significant differences were observed, which need to be studied in detail to understand the underlying mechanisms. To further understand the biology behind observed deviation between hPSC-derived hepatocytes and their *in vivo* counterparts, we performed a Gene Ontology enrichment analysis, and result demonstrates an overrepresentation of genes related to 'transporting processes', 'metabolic processes', 'extracellular matrix', and 'cell-adhesion' among the set of genes with higher expression in the adult liver tissue. This indicates that despite tremendous progress in the field of hepatic differentiation that has been demonstrated the last few years, the differentiation protocols still need to be improved and this has highest priority. We will show characteristics and applications of hESC- and hiPSC-derived hepatocytes, and elaborate on how data from this study can be used. In conclusion, differentially expressed mRNAs and miRNAs between hPSC-derived hepatocytes and adult liver samples has the potential to supply important targets for future more efficient hepatic differentiation procedures.

T-2161

DEVELOPMENT OF NOVEL WEB-BASED REPOSITORIES AND RETRIEVING SYSTEMS THAT AID ANALYSIS OF PUBLIC GENOMIC DATA OF PLURIPOTENT STEM CELLS

Toshiki Taya, Shinya Yamanaka
Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

The stem cell-related public genomic data are rapidly increasing, however, it is not always as easy to review the data because of different formats, nor is it as easy to utilize the data to further advance. We are developing novel web-based repositories with their own data retrieval systems that enable stem cell-related genomic data analysis more easily. We are reconstructing pluripotent stem cells, particularly induced pluripotent stem (iPS) cell-related gene expression data from a public repository of high-throughput genomic data sets. The initial content of the new database include most iPS cell-related expression microarray data which are being re-normalized by us. We are also developing a new means that archives sets of genes reported in publications that serves as a complementary tool to effectively analyze genomic data sets with the system. Since we are constructing not only the databases but also web-based functions to explore the data using query and the data display, researchers with little experience in high-throughput genomic data analysis will benefit from these new systems to observe the landscape of the pluripotent stem cell-related genomic data set. As a result, we then reconstructed publicly available iPS-related gene expression microarray datasets from NCBI GEO. And we then combined a community-based repository to archive sets of genes in publications with the database, both databases are integrated to help query datasets and genes in the reconstructed microarray database. Both are accessible by web browsers. We believe that the databases would achieve our goal to provide and encourage the scientific community to efficiently employ an iPS-related valuable data source.

T-2162

CHARACTERIZATION OF DERIVATION FROM UNDIFFERENTIATION IN COLONIES OF HUMAN INDUCED PLURIPOTENT STEM CELLS BASED ON KINETIC ANALYSIS

Mee-Hae Kim, Eri Masuda, Masahiro Kino-oka
Osaka Univ, Suita, Japan

Cell expansion of human induced pluripotent stem cells (hiPSCs) under undifferentiated state is still challenging because unintentional differentiation occurred during serial subcultures, and the leaving of the undesired differentiation produces the heterotopous cell population, causing the losses of self-renewal and pluripotency for undifferentiated hiPSCs. The deviation of hiPSCs from undifferentiated state, which is called 'de-undifferentiation' in the present study, is a well-known phenomenon accompanying drastical change of cell morphology from a small and cobblestone-like shape to a large and flatten one at the center and periphery of the colonies. In the present study, the de-undifferentiation' in cultures of hiPSCs with SNL and MEF feeder cells was assessed by estimation of hiPSC number at undifferentiated and de-undifferentiated states, and the dynamic variations in their population inside single colonies are compared in cultures between SNL and MEF feeder cells.
The cultures of hiPSCs on a SNL feeder cells were conducted for 144 h. The double immunostainings of all nuclei by DAPI and Oct3/4 expressing nuclei was applied to distinguish undifferentiated and de-undifferentiated states in culture of single hiPSC colonies. The growth rates by replications of undifferentiated and de-undifferentiated cells, and by the transformation were estimated in single colonies which exhibited de-undifferentiation. Based on the kinetic analysis for 144 h of culture time, the specific transformation rate in culture with SNL feeder cells was 7.7 times higher than that with MEF feeder cells, and the specific growth rates for replication of de-undifferentiated cells with SNL and MEF feeder cells were 1.8 and 2.9 times higher than those of undifferentiated cells, respectively. In particular, the specific transformation rate in culture with MEF feeder cells exhibited a negligible level, compared to growth rates for replication of undifferentiated and de-undifferentiated cells, meaning that de-undifferentiated cells drastically expanded through only replication of de-undifferentiated cells. These results concluded that once the deviation occurs in colony, the de-undifferentiated cells make drastic invasion to occupy the colony. From these results, the maintenance of undifferentiated state in subcultures inevitably requires the vigilant care to remove the deviated colonies that include the de-undifferentiated cells.

T-2163

GENERATION AND CHARACTERIZATION OF NON INTEGRATING PATIENT SPECIFIC INDUCED PLURIPOTENT STEM CELLS AT THE CEDARS SINAI IPSC CORE

Anais Sahabian, Loren Ornelas, Austin Hart, Berhan Mandefro, Robert Barrett, Clive Svendsen, Dhruv Sareen
Regenerative Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, United States

The limited availability of human pathological cell types continues to hamper research efforts. This has recently changed by the creation of induced pluripotent stem cells (iPSCs), which provide an abundant source of pathophysiological and patient-specific starting cell material. The first iPSC technology involved the use of integrating viral methods to reprogram the somatic cells that leave a "foot-print" in the genome complicating further studies due to aberrant activation of the integrated transcripts. The Cedars-Sinai iPSC Core generates extensively characterized "foot-print" free iPSC lines from a range of patient somatic tissue.
Fibroblasts or other somatic cell types, including corneal epithelial cells, keratocytes, mammary epithelial cells, lymphoblastoid cells and adipose stem cells, were reprogrammed under 5% O_2 using small molecules and episomal plasmid-based reprogramming factors: *OCT3/4, SOX2, KLF4, L-MYC*, and *LIN28*, and shRNA to p53. Colonies with the best morphology were picked and transferred to BD Matrigel™ Matrix for feeder-free growth in mTeSR®1 medium. For each iPSC line, greater than six clonal lines were generated, cryopreserved, and three clones were further banked and characterized. Pluripotency was confirmed by immunocytochemical staining for surface antigens (SSEA4, TRA 1-81, and TRA 1-60) and nuclear pluripotency antigens (OCT4, SOX2, and NANOG), flow cytometric quantification (OCT4/SSEA4), alkaline phosphatase positive activity, and gene-chip microarray expression and bioinformatics based PluriTest characterization. G-Band karyotyping was performed to ensure normal chromosomal status. Embryonic germ layer gene-specific RT-PCR was performed on spontaneously differentiated iPSCs via embryoid body formation to determine presence of ectoderm, mesoderm and endoderm. To ensure iPSC lines were "footprint-free", RT-PCR and Southern blot analysis of genomic DNA was performed with plasmid-specific primers or probes, while quantitative RT-PCR (qRT-PCR) of mRNA detected any residual transgene expression.
We have successfully generated 34 feeder-free iPSC lines, 26 patient-specific (neurodegenerative, pediatric, inflammatory, skeletal, and eye disorders) and 8 healthy controls. Greater than 80% of iPSC lines (54/68) possessed

normal karyotypes. All iPSC lines generated acquired markers of pluripotency confirmed by various assays and favorably differentiated into the three embryonic germ layers. Flow cytometric quantification revealed all our iPSC lines possess greater than 85% OCT4/SSEA4 double positive cell population. PluriTest gene-chip pluripotency assay passed 88% (43/49) of iPSC lines, while none failed and 12% were listed as “further evaluate”, possibly attributable to disease-specific iPSC lines. Residual transgene expression, by qRT-PCR, was absent at early passages in 69% of iPSCs tested. Lack of episomal plasmid presence in genomic DNA was confirmed in 68% (19/28) of the lines tested. The Southern blot analysis is being performed to exclude any transgene-integration.
The iPSC Core at Cedars-Sinai generates quality non-integrating iPSC cell lines from a variety of tissue sources. Extensive characterization performed on all our iPSC lines demonstrates the rigorous quality control standard that is necessary prior to their distribution to the scientific community for research or testing regenerative therapies.

T-2164

NUCLEAR MIRNAS REGULATE THE METABOLISM OF INDUCED PLURIPOTENT STEM CELLS IN PART THROUGH MITOCHONDRIAL GENES

Man Ryul Lee, Charlie Mantel, Hal E. Broxmeyer
Immunology and Microbiology, Indiana University School of Medicine, Indianapolis, IN, United States

The molecular mechanisms and energy metabolism required
to achieve full reprogramming of somatic cells to induced pluripotent stem
(iPS) cell status are unknown. IPS cells have basic embryonic stem cells
properties, such as self-renewal, pluripotency, and also demonstrate
mitochondria metabolic features that are commonly observed in human tumors. Thus, identification of additional factors required for enhancing efficiency of iPS cell production is important. Using lentiviruses expressing Yamanaka’s factors, we generated iPS cells from CD34+ human umbilical cord blood cells that had been frozen in an unseparated state for more than twenty years. During iPS cell production, we monitored cell surface expression of TRA1-60, a marker for human embryonic stem (hES) cells, within colonies using a live cell staining method. The majority of somatic cells undergoing iPS cell reprogramming accumulate at stable partially reprogrammed stages (TRA1-60 negative cells). We mechanically separated TRA1-60 negative from TRA1-60 positive cells in colonies. Recently, we reported that the miR-302 cluster can promote a transition from incompletely (partial) to fully reprogrammed iPS cells, however, the miRNA functional targets critical for metabolic modification of iPS cells have remained elusive. We hypothesized that some miRNAs can regulate metabolic properties of iPS cells through regulating mitochondrial function. To select specific target genes from partial and fully reprogrammed iPS cells for further study, we explored expression of key factors involved in the regulation of mitochondrial metabolism using miRNA microarrays and mitochondrial metabolism PCR arrays. We found high expression of 5 mitochondrial genes in partially reprogrammed iPS cells including NDUFA1, NDUFA5, SDHA, ATP4B, and COX8C. Bioinformatics analysis and luciferase reporter assay show that miR-31 binds to the 3’UTR of SDHA (luciferase activity decreased 60%). To compare the mitochondrial/metabolic properties between partially and fully reprogrammed iPS cells, we analyzed oxygen consumption rate (OCR) using a metabolic extracellular flux analyzer. The OCR of partially reprogrammed iPS is similar to that of somatic cells, where mitochondrial respiration is increased. This is in contrast to that of fully reprogrammed iPS cells which have lower respiratory rates and increased glycolysis. These results demonstrate that the metabolic signature of fully reprogrammed iPS cells is not identical to that of partially reprogrammed iPS cells. Furthermore, miR-31 may regulate mitochondrial metabolism by suppressing SDHA, known as succinate dehydrogenase complex, subunit A. Our study shows for the first time that miRNAs can influence metabolism of iPS via mitochondrial function and suggest that the efficiency of fully reprogrammed iPS cells might be improved by modification of the miRNA-mitochondria axis.

T-2165

EXAMINING HOW SHATTERED CHROMOSOMES CAUSE INTELLECTUAL DISABILITY USING INDUCED PLURIPOTENT STEM CELLS

Martijn J.E. Kelder[1], Sebastiaan van Heesch[1], Marieke Simonis[1], Koen Braat[2], Evelien Kruisselbrink[2], Wigard Kloosterman[3], Ewart Kuijk[1], Edwin Cuppen[1]
[1]Hubrecht Institute, Utrecht, Netherlands, [A]Department of Cell Biology, [B]Department of Medical Genetics, [2]University Medical Center Utrecht, Utrecht, Netherlands

Induced pluripotent stem cells (iPSCs) are ideal for studying congenital disease in vitro because they have the ability to proliferate indefinitely while maintaining pluripotency. We derived multiple independent iPSC lines from a patient with severe intellectual disability caused by chromothripsis (the complex shattering of chromosomes), and both healthy parents. Chromothripsis is a de novo mutational event that severely impacts on genomic structure and function and has been associated with both congenital disease and cancer. However, the molecular consequences of such complex genomic variations are still poorly understood. The family-based approach presented here provides a very powerful method to study patient phenotypes at the molecular and functional level, where the paternal and maternal iPSC lines function as controls as they represent the non-mutated part of the patient's genome. Thereby we possess the ultimate controls for naturally occuring variation. All iPSC lines have been successfully cultured by manual passaging on feeders and under serum-free and feeder-free conditions using Essential 8 medium. The iPSCs express NANOG and TRA-1-60 indicating faithful reprogramming to pluripotency. Through time lapse DNA, RNA and ChIP sequencing experiments we are currently investigating the impact of chromothripsis on the pluripotent stem cell state, as well as on neural development via established differentiation methods. By exploring differentiation routes towards cell types affected in the patient, we aim to elucidate (part of) the patient's phenotype and improve our understanding on the molecular and mechanistic effects of complex genomic rearrangements during development.

T-2166

THE MECHANISM OF BMP DRIVEN MESENCHYMAL TO EPITHELIAL TRANSITION DURING SOMATIC REPROGRAMMING

Da Liu, Calley Hirsch, Miriam Barrios-Rodiles, Jeffrey L. Wrana
Samuel Lunenfeld Research Institute, Toronto, ON, Canada

Somatic reprogramming proceeds by the induction of the four factors Oct4, Klf4, Sox2 and cMyc (OKSM) that transform differentiated cells into self-renewing, induced pluripotent stem cells (iPSCs). This transformation depends not only on the reprogramming factors, but also the growth factors from the feeder cells and media serum. Using a combination of microarray and functional genomic screens, our lab has previously uncovered three temporal phases of reprogramming, termed initiation, maturation and stabilization. Further we showed a Mesenchymal to Epithelial Transition (MET) in the initiation phase. Here we examine how BMP contributes to early reprogramming and in particular how the BMP signal synergizes with the reprogramming factor Klf4 during MET. Using chromatin immunoprecipitation followed by sequencing (ChIP-Seq), we monitored the dynamic changes in the genome occupancy of the BMP signal transducers, Smad1, 5 and 8 over a timecourse. We observed that MET is initiated with the suppression of key mesenchymal transcription factors Zeb and Snail, followed by the upregulation of notable epithelial genes such as E-Cadherin and Occludin. Moreover we identified that BMP signals do not affect mesenchymal suppression but exclusively modulates the activation of epithelial genes. Using siRNA knockdown, we show that this BMP response during MET depends most critically upon Klf4 rather than other reprogramming factors. Finally we demonstrate a physical interaction between Klf4 and Smad1 and plan to further explore the functional importance of this association. These studies highlight the interplay and mechanistic roles of Klf4 and BMP signaling during MET of somatic cell reprogramming.

T-2167

POTENTIAL OF DERIVING IPS CELLS FROM PERIPHERAL BLOOD T CELLS

Sarah L. Parker[1], Michael C. Jensen[2], Kalia Bared[1], Jakub Tolar[1], Bruce R. Blazar[1]

[1]Department of Pediatrics, Division of Hematology, Oncology, and Blood and Marrow Transplantation, University of Minnesota, Saint Paul, MN, United States, [2]Ben Towne Center for Childhood Cancer Research, Seattle Children's Research Institute, Seattle, WA, United States

Induced pluripotent stem cells (iPSC) have been researched with the hope of understanding early events in development, finding new medications and complementing embryonic stem cells (ESC) for therapeutic applications. Initially derived from fibroblasts, alternative cell types have since been used to generate iPS cells. We proposed to derive iPSC from peripheral blood central memory and effector memory T cells (PBTC) because PBTC are easily isolated and because the resultant iPS cells are expected to maintain the epigenetic memory of a lymphocyte and the T cell receptor (TCR) rearrangement. Thus, the PBTC-iPSC could be expanded *in vitro* and differentiated into professional T cells, yielding a large population with a specific TCR. We isolated PBTC and infected the cells with four standard transcription factors—Oct3, Klf4, Sox2, and c-Myc—with various integrating and non-integrating vectors. For each method, we assessed the feasibility of PBTC transduction using Q-PCR of Oct3, Klf4, Sox2, and c-Myc mRNA 48 hours post-infection. The use of retroviral or lentiviral vectors yielded similar gene expression patterns—a 10-fold increase in the Oct, Sox, and Myc transcripts and 1000-fold upregulation of Klf4 as compared to uninfected T cells. We investigated non-integrating vectors. Utilizing the episomal minicircle vector, we had evidence of nucleofection by flow cytometry but viability post-nucleofection was low. In our studies, another non-integrating vector, the Sendai virus, upregulated the four transgenes to optimal levels for T cell reprogramming. With an MOI of 20, T cells were reprogrammed to iPSC 10 days post-infection with an efficiency of $1x10^{-4}$. Colonies were picked 20 days post-infection and expanded. At passage six, the viability of the colonies was 57% and two colonies were further characterized for the surface markers Tra-1-16, Tra-1-81, SSEA4, and SSEA3 as well as the intracellular markers Nanog and Oct4 by immunostaining. Gene expression was simultaneously measured by Q-PCR and showed an increase by 1,000-10,000 fold in the Oct4, Sox2, and Nanog transcripts. Lin28, another key protein regulating pluripotency, was amplified 100,000-fold when compared to mature T cells. We characterized the methylation status of the key pluripotency promoters Oct4 and Nanog by bisulfite sequencing. Whereas CpG islands were methylated in these promoters in mature T cells, the same CpG islands were unmethylated in our PBTC-iPSC. Experiments redifferentiating these PBTC-iPSC are in progress and will provide insights into the retention of epigenetic and TCR rearrangements that persist during reprogramming into iPSC and differentiation into T cells. Here, we investigated the feasibility and efficiency of reprogramming with various methods in PBTC with the rationale that PBTC-iPSC are a possible unlimited source of T cells with defined specificity which are amenable to gene modification, though future studies are required to optimize conditions for therapeutic intent.

T-2168

GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS FROM HUMAN MESENCHYMAL STEM CELLS BY RECOMBINANT PROTEINs

Kou-Juey Wu[1], Tsung-Chi Lu[2], Wen-Mei Kung[2], Yen-Chao Su[3], Chi-Hung Huang[2]

[1]Biochemistry & Mol. Biology, National Yang-Ming Univ., Taipei, Taiwan, [2]Stem Cell Division, Taiwan Advance Biopharm, Inc., New Taipei City, Taiwan, [3]Biochemistry, National Chung-Hsing Univ., Taichung, Taiwan

Induced pluripotent stem (iPS) cell technology has enormous potential in regenerative medicine. However, most methods utilized to generate iPS cells require the use of genetic materials (DNA, microRNA, etc) that may induce genetic mutations. Therefore, a safe iPS cell manufacturing technology for clinical application of iPS cells is essential. Here we report the generation of stable iPS cells from human mesenchymal stem cells (MSCs) by directly delivering two purified recombinant reprogramming proteins (Oct4 and Sox2) that were fused with a nuclear localization signal peptide (NLSP) named TAT. We purified TAT fusion proteins, TAT-Oct4 and TAT-Sox2, in large scales and established an iPS conversion protocol. Two iPS clones were obtained using this iPS conversion protocol and they showed high similarity to the human embryonic stem cells (hESCs) from the aspects of morphology, proliferation, and expression of most characteristic pluripotency markers. The iPS clones produced from this protocol successfully differentiated into three embryonic germ layers both in vitro and into teratomas. This method eliminates the

potential risks that the conventional methods may induce to cause genetic mutations or carcinogenesis. Our iPS conversion protocol could potentially provide a safe source of patient-specific iPS cells for clinical trials and significantly contribute to regenerative medicine in the future.

T-2171

PERFORIN POSITIVE DENDRITIC CELLS FOR MITIGATING THE IMMUNOGENICITY OF EPS AND IPS DERIVED TISSUE

Yael Zlotnikov Klionsky, Lior Zangi, Liran Yarimi, David Hagin, Esther Bachar-Lustig, Bar Nathansohn-Levi, Yair Reisner
Immunology, Weizmann Institute of Science, Rehovot, Israel

Although marked progress has been achieved in differentiating embryonic pluripotent cells (EPS) into various cell types of therapeutic value, their immunogenicity represents a major challenge. This concern seemed to be addressed with the advent of induced pluripotent stem cells (iPS). However, very recent studies have indicated that potential immune barriers might still be present even when using iPS derived cells. This unexpected problem, although controversial, might be related to imperfect differentiation that could potentially lead to presentation of embryonic antigens on iPS derived tissues. One approach to address the immunogenicity of EPS or iPS could be offered by induction of specific immune tolerance to donor antigens using tolerogenic cells derived from the same donor. Ideally, this could be afforded by EPS or iPS derived hematopoietic stem cells (HSC), capable of inducing permanent central tolerance through their continuous presence in the host thymus. However, attempts to develop protocols for effective generation of HSC from embryonic stem cells has faced many difficulties. Thus, investigation of other tolerogenic cell types is warranted.
Recently, we have described a unique perforin positive tolerogenic population of DCs (p-DCs). We generated these highly defined p-DCs from hematopoietic progenitors and characterized in-vitro the mechanism/s mediating deletion of alloreactive TCR-transgenic or syngeneic antigen-specific CD4+ and CD8+-T cell upon encountering their cognate antigen on the p-DCs. While CD4+T-cells were deleted non-specifically through the NO system, CD8+ T-cell deletion was found to be antigen-specific, through the release of perforin/GranzymeA granules highly expressed in the p-DCs. Likewise, when tested in-vivo, these p-DCs were able to delay T-cell mediated BM allograft rejection through an MHC-dependent mechanism.
Next, to further enhance the tolerogenic efficacy of p-DC we attempted to optimize a protocol for arresting the cells in their immature state. To that end, we have compared several treatment modalities and optimal results were attained by ex-vivo treatment with aspirin. Thus, aspirin-treated p-DCs were found to be resistant to LPS-induced maturation while maintaining their killing capacity.
It has been recently demonstrated that immature myeloid DCs can be generated ex-vivo from EPS or iPS. Therefore, our results strongly suggest that EPS or iPS derived p-DC, could potentially offer a new strategy for the induction of tolerance towards other tissues derived from these pluripotent stem cells, and pave the way for their wide use in regenerative medicine.

T-2172

EXTRACT OF INDUCED PLURIPOTENT STEM (IPS) CELLS REPROGRAMS ADULT SOMATIC CELLS INTO SECONDARY IPS CELLS WITH IMPROVED EFFICIENCY AND SAFETY THROUGH ZSCAN4

Yoo-Wook Kwon, Jae Seung Paek, Hyun-Jai Cho, Han-Mo Yang, Sahmin Lee, Ho-Jae Lee, Ju-Young Kim, Jooeun Lee, Hyo-Soo Kim, Young-Bae Park
Innovative Research Institute for Cell Therapy, Seoul National Univ Hospital, Seoul, Korea, Republic of

Previously, we found that the delivery of ES cell-derived proteins enables the full reprogramming of adult fibroblasts (protein-iPS cells), converting them into pluripotent stem cells without transduction of defined factors. During the reprogramming, global gene expression and epigenetic status including DNA methylation and histone modifications convert from somatic to ES-equivalent status. ES cell extract derived-iPS cells are biologically and functionally indistinguishable from ES cells in potentiality of *in vitro* and *in vivo* differentiation. Furthermore these cells

show complete developmental potency. However, the efficiency to generate iPS with ES cell extract is still low. Here, we demonstrated that proteins derived from iPS cells, which were previously generated by ES cell extract, reprogrammed somatic cells to ES like cells so called ‘the secondary protein-iPS cells’ and we confirmed fetal animals (E12.5) can be derived from these cells**.** Surprisingly, the efficiency to form Oct4 positive colonies was remarkably improved by treatment of somatic cells with iPS cell extract compared to ES cell extract. Through screening the differentially expressed genes between iPS and ES cells, Zscan4, known as enhancing telomere elongation and stabilizing genomic DNA, was picked as strong candidate to promote efficiency of reprogramming. Interestingly, protein extracted from Zscan4-overexpressed mES cells enhanced formation of Oct4 positive colonies. Our results provide an efficient and safe strategy to reprogram somatic cells by using iPS cell extract and Zscan4 might be a key molecule to improve reprogramming efficiency.

T-2173

IN VITRO DIFFERENTIATION OF ENDOTHELIAL PROGENITOR CELLS GENERATED FROM MOUSE INDUCED PLURIPOTENT STEM CELLS

Mohammad Reza Dastouri[1], Aynur Karadağ[2], Deniz Balcı[1], Günseli Çubukçuoğlu Deniz[1], Serkan Durdu[3], Ahmet Rüçhan Akar[3], Alp Can[4]

[1]Department Of Stem Cell, Ankara University Biotechnology Institute, Ankara, Turkey, [A]Department of Medical Biology, [B]Department of Cardiovascular Surgery, Heart Center, [C]Department of Histology & Embryology, [2]Ankara University School of Medicine, Ankara, Turkey

End-term differentiated somatic cells can be converted to a pluripotent state through transduction of four transcription factors, namely Oct4, Klf4, Sox2 and cMyc. These induced pluripotent stem cells (iPS cells) resemble embryonic stem cells in many aspects while avoiding general ethical concerns. Autologous iPS cells after reprogrammed into endothelial progenitor cell (EPC) may offer several advantages in the treatment of cardiovascular disorders because of their cardiogenic and vasculogenic differentiation potential. To reach that purpose, we reprogrammed and then characterized mouse fibroblast-driven iPS cells into Flk-1/KDR^{+} (vascular endothelial growth factor receptor-2), a well-recognized EPC marker. Further maturation of EPC was characterized by the expression of CD31 and VE-cadherin, both of which are well-known endothelial cell-specific adhesion molecules.

Puromycin-resistant iPS cells (Ng-20D-17) were expanded in culture on the mouse embryonic fibroblasts (MEFs) and then purified from MEFs. Purified iPS cells were differentiated into Flk-1^{+} cells with the use of differentiation medium (α-minimum essential medium supplemented with 10% fetal calf serum and 5×10^{-}5 M 2-mercaptoethanol) in the absence of leukemia inhibitory factor (LIF) on type IV collagen-coated dishes. We then analyzed Flk-1 gene expression and protein levels with quantitative real-time PCR (qRT-PCR), Western blot and immunocytochemical methods on days 2, 3, 4 and 5. Morphological changes were evaluated during differentiation process using confocal and scanning electron microscopy. As a first step, Flk-1 expressing cells were selected by fluorescence activated cell sorting (FACS) in each culture day. In the second step, FACS-purified Flk-1^{+} cells were cultured on type IV collagen-coated dishes in differentiation medium with 100ng/mL human $VEGF_{165}$ (vascular endothelial growth factor) to induce EPC formation. On day 2 and 3 following induction, CD31 and VE-cadherin gene expression and protein levels were analyzed with qRT-PCR, Western blot and immunocytochemical methods. As a result of the first step we found that Flk-1 expressing cell number reached to a peak level (24%) on day 4 followed by a progressive decline subsequently. In the second step, CD31 and VE-cadherin positive cells were generated and enriched during day 2-3 of induction. We concluded that optimal time for harvesting Flk-1^{+} cells on by FACS was is day 4 of initial differentiation. Following isolation of Flk-1^{+} progenitor cells they were are further matured into functional EPCs by VEGF165 within 2-3 days of induction.

In conclusion, we showed that EPCs could be successfully derived from mouse fibroblast-driven iPS cells. iPS cells may therefore play be used in an important role in the treatment of heart failure, ischemic heart disease, and critical limb ischemia by remodeling the blood vessels and could be considered for an in vivo model for the translational research.

T-2174

COMPARISON OF HEMATOPOIETIC DIFFERENTIATION EFFICIENCY AMONG MANY IPS CELL LINES

Masatoshi Nishizawa[1], Kazuhisa Chonabayashi[1], Akiko Oishi[1], Ikue Takei[1], Misato Nishikawa[1], Akifumi Takaori-Kondo[2], Shinya Yamanaka[1], Yoshinori Yoshida[1]

[1]Department of Reprogramming Science, The Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [2]Hematology and Oncology Department, Graduate school of Medicine, Kyoto University, Kyoto, Japan

Hematopoietic differentiation from human pluripotent stem cell (PSC) attracts much attention due to its huge potential for regenerative medicine or as an excellent tool in hematological research. Thus far, not a few papers have reported about efficiency of hematopoietic differentiation from human PSCs. Although some papers reported about presence of epigenetic memories of human induced pluripotent stem (iPS) cells, the amount of influence of epigenetic memory of human iPS cell on differentiation efficiency is still to be known. One of major limitations of earlier studies is that the number of PSC lines utilized in the studies was limited. Furthermore, especially in studies using human PSCs, genetic difference among individual donors of iPS cells seems to be large, thus the study using many PSC lines from many donors is warranted.

To address these issues, we planned to investigate hematopoietic differentiation potential of many iPS cell lines derived from several kinds of somatic cells. We differentiated 39 iPS/ES lines (iPS 35 lines, ES 4 lines) into blood cells via embryo body forma-

tion without feeder cells, and compared differentiation efficiency by counting generated CD43 positive cells 15 days after start of differentiation. The iPS cell lines utilized in this study included iPS cells which were derived from dermal fibroblast (HDF-iPS; n = 16), bloods (Blood-iPS; n =13) (including cord blood (n = 3) and peripheral blood (n = 10)), keratinocytes (Keratinocyte-iPS; n = 3) , and dental pulp cell (DP-iPS; n = 3), and were generated by using retrovirus vector (n = 9), episomal vector (n = 25), and Sendai virus vector (n = 1). Differentiation efficiency was HDF-iPS: 0.56 ± 0.37, Blood-iPS: 0.67 ± 0.57, Keratinocyte-iPS: 0.86 ± 0.42, DP-iPS: 0.67 ± 0.35, and ES:1 ± 0.54 (normalized to ES mean = 1), and there was no significant difference. We have also found that there was much variation even among iPS cells derived from same somatic origin and generated by same method.
Our data suggest that finding the optimal cell lines to produce blood cells efficiently is useful for application of the derived blood cells from iPS cells.

T-2175

IPS CELLS GENERATION-ASSOCIATED POINT MUTATIONS

Ryoko Araki, Mayumi Sugiura, Yasuji Kasama, Misato Sunayama, Masahiro Uda, Miki Nakamura, Shunsuke Ando, Yuko Hoki-Fujimori, Masumi Abe
National Institute of Radiological Sciences, Chiba, Japan

To address the issue whether point mutations occur associated with induced pluripotent stem cells (iPSCs) generation or are already existed in the parent somatic cells (pre-existing), we studied the point mutations in mouse iPSC lines. To achieve the end, we attempted to establish iPSCs from the mouse embryonic fibroblasts (MEF) of an inbred mouse strain, C57BL/6, with plasmid vectors, and successfully established 35 of iPSC lines. Then 6 out of the 35 were revealed to be integration-free. In this study, we conducted whole genome sequencing on the three such integration-free iPSC lines and their parent somatic cells. We prepared each parent MEF fraction from independent single embryo, because substantial numbers of intra-strain single nucleotide polymorphisms (SNPs), which could be false positives of point mutations, exist even in inbred mouse. Our sequencing achieved 55% of entire genome with more than 10 redundancies, and identified ~300 point mutations in each genome. Sanger sequencing on the 90 single nucleotide variants (SNVs) randomly chosen from the point mutation candidates verified our approach; 90/90 (100%) were observed only in iPSCs genomes but not in their parent somatic cells. In addition, no common SNV was observed between different iPSC lines. Thus, our results using three independent iPSC lines are consistent with the previous studies on both human and mouse iPSC genomes.
Focusing on an iPSC line, 2A-4F-136, we attempted to address the issue when the point mutations arose in their genomes. First, although we performed ultra-deep sequencing (~10 million reads for each SNVs in their parent genome) to evaluate whether theses SNVs were existed already in their parent somatic cells or not, very few pre-existing SNVs were detected. Next, we conducted variant allele frequency test on 43 SNVs that were randomly selected, then, in addition to around 50% frequency, we also observed a considerable number of SNVs at less than 50% allele frequency, strongly suggesting a presence of 25% and 12.5% allele frequency point mutations. To ensure our observation, we conducted more whole genome sequencing up to 21 redundancies. A clear peak was appeared around 25% allele frequency in addition to the 50% frequency; 105 SNVs candidates were observed around at 25% frequency. Additional variant allele frequency test by amplicon sequencing on the 46 out of the 105 SNVs clearly confirmed that these are not 50% SNVs, though 2 out of them exhibited 50% frequency. 25 of them were around at 25% frequency and the remaining 19 were detected less than 25% frequency. The intensity of Sanger sequencing also suggested 25% or less frequency for these SNVs candidates. Similar results were observed another integration-free iPSCs, clone 2A-4F-118. Lastly, we examined three iPSC lines, one was genome integration-free line and two lines that had been established using retrovirus vector system, and revealed considerable numbers of 25% SNVs. Since pre-exising SNVs must be observed as 50% allele frequency, our observation strongly suggested that substantial numbers of point mutations occur during the conversion process, especially in their initiation steps, of iPSC generation.

T-2176

DPPA3 BINDS TO THE IG-DMR OF THE DLK1-DIO3 IMPRINTING CLUSTER AND PREVENTS ITS IMPRINTING LOSS DURING IPS CELL GENERATION.

D. V. Krishna Pantakani[1], Xingbo Xu[1], Toshinobu Nakamura[3], Lukasz Smorag[1], Ralf Dressel[2], Antje Fitzner[4], Matthias Linke[4], Ulrich Zechner[4], Wolfgang Engel[1]

[A]Institute of Human Genetics, [B]Department of Cellular and Molecular Immunology, [1]University of Goettingen, Goettingen, Germany, [2]Department of Pathology, Osaka University, Osaka, Japan, [3]Institute of Human Genetics, Johannes Gutenberg-University Mainz, Mainz, Germany

Dppa3 binds to the IG-DMR of the *Dlk1-Dio3* imprinting cluster and prevents its imprinting loss during iPS cell generation.

D.V. Krishna Pantakani[1], Xingbo Xu[1], Toshinobu Nakamura[2], Lukasz Smorag[1], Ralf Dressel[3], Antje Fitzner[4], Matthias Linke[4], Ulrich Zechner[4] and Wolfgang Engel[1]

[1]Institute of Human Genetics, University of Goettingen, Heinrich-Dueker-Weg 12, 37073 Goettingen, Germany. [2]Department of Pathology, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan. [3]Department of Cellular and Molecular Immunology, University of Goettingen, Humboldtallee 34, 37073 Goettingen, Germany. [4]Institute of Human Genetics, Johannes Gutenberg-University Mainz, Langenbeckstrasse 1, 55101 Mainz, Germany.

Reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) often results in defects at the genetic and epigenetic level. Of particular interest is the loss of imprinting at the paternally imprinted *Dlk1-Dio3* cluster in a majority of the iPSC clones, which ultimately fail to show full pluripotent cell characteristics. In the present study, we analyzed the expression of germ cell (GC) marker genes (*Blimp1*, *Fragilis*, and *Dppa3*), that have been described to become activated early during somatic cell reprogramming. We found that Dppa3, the guardian of the maternal genome and imprinted loci that protects them against DNA demethylation during early embryonic development, was present only in iPSC lines showing expression of *Gtl2* (*Gtl2*^{on}), an indicator of normal imprinting at *Dlk1-Dio3* cluster, but not in iPSCs with loss of imprinting (*Gtl2*^{off}). Subsequently, we found that exogenous Dppa3 together with classical reprogramming factors can efficiently reprogram somatic cells into iPSC clones that all display normal imprinting at *Dlk1-Dio3* region. Next, we performed chromatin immunoprecipitation assays with a Dppa3 antibody on embryonic stem cell (ESC) chromatin and found Dppa3 to be associated with a specific region within the intergenic differentially methylated region (IG-DMR) of *Dlk1-Dio3*. Further studies indicated that the forced expression of Dppa3 during reprogramming leads to the maintenance of imprinting at this cluster by counteracting and reducing the binding of Dnmt3a, a de novo DNA methyltransferase that establishes DNA methylation. Collectively, our results show that *Dppa3* is a genetic factor necessary for preventing abnormal imprinting at the *Dlk1-Dio3* region during somatic cell reprogramming.

T-2177

GENERATION AND CHARACTERIZATION OF MESENCHYMAL STEM CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS IN VIVO AND IN VITRO

Takeshi Teramura
Kinki Univ, Osaka-sayama, Japan

Objective:
Mesenchymal stem cells (MSCs) are a promising cell source for regenerative medicine. However, factors such as donor age, patient background, and cellular senescence during expansion can limit their therapeutic potential. In this study, we derived MSCs from human iPSCs in vivo and in vitro and examined their characteristics in detail.
Methods:
Induction of MSCs in vivo (in vivo-MSCs): Human 253G1 iPSCs were encapsulated in Matrigel and transplanted into SCID mice. After 6-weeks, the transplants were recovered as teratomas, dissociated with collagenase and sorted for CD29/CD105 by FACS. Induction of MSCs in vitro (in vitro-MSCs): Embryoid bodies (EBs) were formed from 253G1

iPSC suspension cultures. The EBs were then seeded onto gelatin-coated dishes and cultured for 14 days under hypoxic conditions in bFGF-supplemented 10%FCS-alphaMEM (as reported for rabbit ESCs; Teramura et al., Cell Transplant. 2012. Epub ahead of print). Candidate CD29/CD105-positive cells were collected by FACS. Two normal human MSC lines (native-MSCs) were used as controls.
Results:
Fibroblastic cells were obtained using both methods. These cells expressed the MSC-markers CD29, CD44, CD73, CD90, CD105, CD106 and Vimentin, and could differentiate to multiple mesenchymal lineages (osteoblasts, chondrocytes and adipocytes). In vitro-MSCs proliferated at a similar rate to native-MSCs and became senescent within 8 passages. Teratomas did not form after in vitro-MSCs were transplanted into SCID mice. In vivo-MSCs, however, were highly proliferative, had high telomerase activity and frequently formed teratomas. In addition, re-expression of exogenous reprogramming genes was observed for in vivo-MSCs.
Conclusion:
MSC-like cells can be obtained from human iPS cells both in vivo and in vitro. In vitro-MSCs possess closed characteristics that are similar to native-MSCs. These cells are also limited in terms of their proliferation. In vivo-MSCs, on the other hand, are highly proliferative and tumorigenic. These results suggest that iPSCs can generate different types of MSCs based on the conditions used for MSC induction. It is clear that both in vivo-MSCs and in vitro-MSCs possess specific advantages that warrant further investigation. In the future, a detailed molecular comparison of both types of MSCs may lead to an improved induction method for producing MSCs from iPSCs.

T-2178

USING HUMAN IPS-DERIVED CARDIOMYOCYTES TO STUDY CARDIOMYOPATHY IN MUSCULAR DYSTROPHIES

RADBOD DARABI[1], Jakub Tolar[2], Rita C.R. Perlingeiro[1]
[A]Medicine, [B]Pediatrics, [1]UNIVERSITY OF MINNESOTA, Minneapolis, MN, United States

Cardiac muscle is affected in many forms of muscular dystrophies (MDs) including dystrophin-associated disorders such as Duchene Muscular Dystrophy (DMD). This pathology often leads to severe life threatening cardiac complications. With the advancement of respiratory care, many DMD patients have a chance to survive to the 3rd decade of life which by this time most cases display cardiomyopathy. In DMD, dystrophin deficiency leads to sarcolemma fragility, calcium leakage and other pathologies which eventually lead to skeletal and cardiac cell damage.
The technology of cell reprogramming, which allows for derivation of patient specific induced pluripotent cells (iPS cells), creates a great opportunity for in vitro disease modeling as well as drug screening. Therefore, in this study, we analyzed DMD iPS-derived cardiomyocytes to model and evaluate pathologic mechanisms involved in DMD cardiomyopathy.
By using an efficient in vitro cardiomyocyte differentiation method, we have succeeded in differentiating control (unaffected) and DMD iPS cells into cardiomyocytes in a reproducible and efficient manner. Our results confirmed the presence of DMD pathology in DMD iPS-derived cardiomyocytes when compared to their control counterparts. Structural abnormalities in intercellular cardiomyocyte adhesions, beating rates and cardiomyocyte sarcomeric alpha actinin distribution were detected in DMD but not in control human iPS cells during cardiac differentiation. Time-course FACS analysis studies also revealed increased Annexin V positive population during in vitro cardiomyocyte maturation in DMD but not in control human iPS cells. This finding was accompanied by increased levels of cleaved caspase-3 expression.
These findings suggest increased apoptosis in DMD iPS-derived cardiomyocytes may represent as one of the involved mechanisms in DMD cardiomyopathy. Further analyses include measuring intracellular ROS activity, comprehensive gene expression assays, action potential measurements using patch clamp technique and calcium current imaging which will provide more insights toward DMD cardiomyopathy. The final results of this study will provide a platform for future assessment of any treatment (i.e. pharmacologic or gene correction approaches) on DMD iPS-derived cardiomyocytes.

T-2182

IPS-BASED MODELING OF NIJMEGEN BREAKAGE SYNDROME

Barbara Mlody[1], James Adjaye[2]
[1]*Vertebrate Genomics, Max-Planck Institute for Molecular Genetics, Berlin, Germany,* [2]*Stem Cell Research and Regenerative Medicine, Heinrich Heine University, Düsseldorf, Germany*

Nijmegen breakage syndrome (NBS) is a rare, autosomal, recessive genetic disorder first described in 1981 in patients living in Nijmegen, Holland. NBS patients display a characteristic facial appearance, microcephaly and a range of symptoms including elevated sensitivity to ionizing radiation, chromosome instability, a high frequency of malignancies, accelerated shortening of telomeres, abnormal cell cycle checkpoints, growth retardation and immunodeficiency. The clinical features overlap with those of Ataxia-telangiectasia, Ligase IV syndrome, Non-homologous end-joining factor 1 (NHEJ1) syndrome and Fanconi anemia.
The NBN protein, Nibrin, is part of a DNA repair, DNA double-strand break (DSB) sensor and damage signaling system and forms a trimeric complex together with MRE11 and RAD50. Mutations in this gene result in truncated proteins that maintain part of their function, however null mutation of the homologous gene in mice is lethal.
DSBs, the main target for NBN, result from exogenic stresses like ionizing radiation (IR) and chemical compounds or from endogenous mechanisms like reactive oxygen species (ROS), stalled replication forks and recombination in cells of the immune system. As ROS are generated constantly as by-products of mitochondrial respiration, ROS may probably be detrimental for NBS cells under physiological conditions. Interestingly, pluripotent cells, both ESCs and iPSCs rely on glycolysis rather than oxidative phosphorylation as the main source of energy (Prigione 2010, stem cells). Based on this finding, we hypothesized that inducing pluripotency in NBS fibroblasts might by-pass ROS-mediated genome instability. To test this, we performed the following, a) Reprogrammed fibroblasts, derived from NBS patients, into iPS-cells (NBS-iPSC) as an in vitro model of the disease. b) Compared the transcriptomes of four NBS patient derived dermal fibroblasts to healthy foreskin fibroblasts (HFF1) in order to uncover molecular features and etiology of the disease. c) Compared the transcriptomes and stress responses of undifferentiated ES cells (H1 and H9), NBS-iPSCs and HFF-iPSCs.
Our findings are: All iPSCs expressed pluripotency associated proteins (Alkaline phosphatase, OCT4, NANOG, TRA1-81, TRA1-60, SSEA4) and pluripotency was further confirmed both in vitro (EB assays) and in vivo (teratoma formation). Comparative transcriptome and associated pathway analyses revealed (a) that NBS fibroblasts compared to healthy HFF1 seem to have a high impact on cell cycle regulation, apoptosis, p53 signalling and the Fanconi Anemia pathway. (b) The comparisons between ES cells, HFF-iPSCs and NBS-iPSCs revealed regulated genes and pathways associated with DNA replication, glycolysis, pyrimidine, fructose and mannose metabolism as well as DNA repair related pathways. Interestingly all of these pathways are known to be associated with ROS homeostasis. (c)NBS-iPSCs retained a set of genes related to B cell receptor signaling pathway, ion transport, cell adhesion and others, not seen in ESCs and HFF-iPSCs.
Comparative tests based on sensitivity towards oxidative stress and DNA damaging agents such as hydrogen peroxide and bleomycin, revealed that NBS-iPSCs and NBS-fibroblasts compared to healthy HFF1 were highly sensitive to DSB inducer bleomycin but similar sensitive towards oxidative stress induced by exogenous hydrogen peroxide.

T-2183

ARE NEURAL CREST STEM CELLS AN OPTIMAL SUBSTRATE FOR REPROGRAMMING?

Ahmed M. Fahmy, Irina Brokhman, Derek van der Kooy
Molecular Genetics, University of Toronto, Toronto, ON, Canada

The discoveries made by Yamanaka et al and other groups in reprogramming somatic cells either to pluripotent stem cells (called induced pluripotent stem cells, or iPSCs) or directly to other differentiated cells has opened up a new field in stem cell research. However these reprogramming techniques are plagued by problems relating to the efficiency of the process and the poor understanding of their cellular mechanism. These are some of the factors preventing practical application in the clinical field. It is well established that multipotent neural crest stem cells (NCSCs) migrate to many parts of the developing embryo where they can produce a vast array of cell types, some

of these NCSCs remain undifferentiated until adulthood. We hypothesise that a primary source of iPSCs and directly reprogrammed cells are NCSCs found in culture. We traced the lineage of neural crest cells in E15 mice embryos using a Wnt1-Cre driver crossed to a ROSA-YFP reporter containing a floxed stop codon. Wnt1 is expressed in the neural plate border between what will become the neural tube and the ectoderm. Its expression specifies the dorsal tip of the neural tube to become the neural crest (NC) and is continually expressed until shortly after delamination and migration of the NCSCs. We assayed head and trunk fibroblast samples and found that YFP positive cells (Wnt1 and NC derived) were present in primary culture and they increased in proportion with passage number. This shows that there exists neural crest derived cells in cultures similar to those used in reprogramming studies. We reprogrammed a skin sample of mouse embryonic fibroblasts (MEFs) and found that 90% of the iPSCs produced were YFP positive (from NC origin). Furthermore YFP negative cells from skin of the head (non-NC derived) were incapable of being reprogrammed to iPSCs suggesting that the only cells in MEF samples capable of producing iPSCs are of NC origin. Further experiments will look at which subpopulation of the YFP cells are responsible for the production of iPSCs. Other experiments will be testing this hypothesis in direct reprogramming experiments of hepatocytes to neurons. These studies can help better understand the cellular mechanism of reprogramming and will help devise more efficient techniques of reprogramming.

T-2184

MODELING NEURODEVELOPMENTAL DEFECTS IN MOWAT WILSON SYNDROME USING IPSCS

Anjana Nityanandam, Kristin Baldwin
Molecular and Cellular Neuroscience, The Scripps Research Institute, La Jolla, CA, United States

An increasing number of neurodevelopmental and psychiatric disorders are being associated with heterozygosity, and possible haploinsufficiency, in key neural developmental genes. Such disorders often cause severe mental retardation, and are detrimental to normal growth and development. The etiology of these diseases has been studied thus far using animal models. However, rodent brains differ significantly from human brains and their neurons develop along different timescales and in different locations. This project is aimed at developing a human in vitro model of Mowat- Wilson Syndrome (MWS), a rare disease associated with mild to severe mental retardation, facial dysmorphology, microcephaly, delayed motor and speech development, and seizures. All reported MWS patients carry mutations in the transcriptional repressor Sip1/Zfhx1b. Mouse models of MWS show impaired temporal sequence of cortical neurogenesis resulting in disrupted proportion of neurons and premature gliogenesis. To model MWS, we used two approaches. First we established iPSCs from patient and control fibroblasts using modified mRNAs, and differentiated them towards a neural lineage using multiple methods. Second, to account for the interline variability in the neurogenic potential of iPSC lines, we are comparing isogenic lines in which we have used shRNA to reduce Sip1 expression. Our preliminary studies suggest that MWS iPSCs have an increased tendency to generate the Otx2+ subtype of neural progenitor cells, corticofugal projection neurons and subtypes of striatal neurons, at the expense of other cortical and striatal cell types. These data suggest that it is possible to model a monogenic neurodevelopmental disorder with iPSCs using a combination of isogenic and patient-specific lines and establish a system to search for factors to restore normal neurogenesis to affected individuals.

T-2185

AUTOLOGOUS TRANSPLANTATION OF INDUCED PLURIPOTENT STEM CELL-DERIVED NEURONS CAUSES MINIMUM IMMUNE REACTION IN THE BRAIN OF NON-HUMAN PRIMATE

Asuka Morizane[1], Daisuke Doi[1], Tetsuhiro Kikuchi[1], Keisuke Okita[1], Akitsu Hotta[1], Toshiyuki Kawasaki[2], Hirotaka Onoe[2], Takashi Shiina[3], Jun Takahashi[1]
[1]CiRA, Kyoto University, Kyoto, Japan, [2]Center for Molecular Imaging Science, RIKEN, Kobe, Japan, [3]Dep. Basic Medical Science and Molecular Medicine, Tokai University School of Medicine, Isehara, Japan

[Background]

Pluripotent stem cells have promise for serving as a source of donor cells for cell transplantation. Especially induced pluripotent stem cell (iPSC) technology has potential to prepare the donor cells from a patient's own somatic cells that would cause minimal immune reaction after transplantation. On the other hand, brain is considered as immunologically privileged site and fetal mesencephalic grafts survive in allo-transplantation with or without immunosupression.
[Materials & Methods]
We generated several lines of primate iPSCs by retroviral system or episomal vector system. After one month's differentiation into neural cells with dopaminergic phenotype, we transplanted them back to the striatum of the original monkeys (auto) or to that of the other ones (allo). We monitored the graft survival and immune reaction by magnetic resonance image (MRI) and positron emission tomography (PET) until three months. Finally we sacrificed the animals and analyzed the brain slices histologically.
[Results]
Dopaminergic neurons survived in all the grafts including allo-grafts. There was no significant difference in the graft volume between auto- and allo- grafts. The number of surviving dopamine neurons was higher in the auto-grafts. There were invading CD45-positive leukocytes more in the allo-grafts than the auto-grafts. The activation of the resident microglia positive for Iba-1 and MHC class-2 was also observed to a higher extent in the allo-grafts.
[Discussion]
While allo-grafting has the risk of strong immune reaction, auto-grafting causes minimum immune reaction and results in the sufficient number of the survived neurons. On the other hand, there were some cases of allo-grafts that caused little immune reaction suggesting that it would also be useful in the future if an appropriate cell line could be selected before transplantation. Although auto-grafting with own iPSCs is the best strategy in theory, it will cost much in practice. Establishment of master stock of iPSCs with different types of human leukocyte antigen (HLA) and the HLA-matched grafting using the iPSCs from the stock could be another option in the future.

T-2186

DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO NEURAL PROGENITOR CELLS AND NEURONS ON MICROFLUIDIC CHIPS

Nianzhen Li[1], Lukasz Szpankowski[1], Brian Fowler[1], Naga G. Devaraju[1], Anne Leyrat[1], Michael Noris[1], Myo Thu[1], Michael Wong[1], Christian Johonson[1], Jay West[1], Shuyuan Yao[2], Marc Unger[1], Robert C. Jones[1]
[1]Fluidigm Corporation, South San Francisco, CA, United States, [2]Allen Institue for Brain Science, Seattle, WA, United States

Since *in vivo* cell fate determination and cell-cell signaling involve multiple genes, extracellular factors, and intracellular signaling pathways, finding the right combinations for *in vitro* cell culture conditions is invaluable for studying fundamental cell biology questions and for developing cell-based therapies. However, using traditional cell culture systems, multi-factorial experiments are often laborious and difficult to reproduce. Microfluidic technologies allow for precise control of microenvironment of cells, facilitate studies of multi-factorial combinations, and enable development of robust, reproducible, and chemically-defined cell culture systems. We have designed and fabricated a prototype microfluidic chip and an automated instrument that can culture cells on chip for extended periods of time and deliver multiple combinations of different factors to cells. Each chip includes 32 cell culture microchambers and 32 reagent inlets. Reagents can be automatically multiplexed to desired concentrations and combinations at various pre-defined time points. Cells can be cultured and stained on chip, or harvested from the chip for continued off-chip culturing, single-cell genomic analysis, and/or functional assays. We have demonstrated growing human induced pluripotent stem cells (hiPSCs) on chip for over a week; the hiPSCs retain pluripotency markers and can form colonies. The hiPSCs can also be differentiated on chip to neural progenitor cells or nociceptor neurons by scheduled combinatorial dosing of small signaling molecules. The identities of cells at different stages were profiled with immunostaining and single-cell gene expression analysis of targeted genes. The results were consistent with published reports and were confirmed in large well-dish format. In summary, the prototype automated microfluidic system reported here provides the potential to study and screen the precise combinatorial effects of multiple

factors on stem cell culture maintenance, reprogramming, and differentiation–and therefore could be a valuable tool for the stem cell community.

T-2187

DEVELOPMENT OF PORCINE CLONED EMBRYOS USING INDUCED PLURIPOTENT STEM-LIKE CELLS

SuJin Kim, Joon Ho Moon, Hee Sun Kwon, Dae Kee Kwon, Bego R. Torre, Sol Ji Park, Ok Jae Koo, Jung Taek Kang, Ji Yei Choi, Byeong-Chun Lee, **Goo Jang**
College of Veterinary Medicine, Seoul National University, Seoul, Korea, Republic of

Pig models are important for the study of human disease or cell therapy. To produce these models, developing gene modified cells or pigs have been hampered because there is no germ line transmissible to embryonic stem cell line in pig. Recent emerging tool, induced pluripotent stem (iPS) cells have enabled us to give a hypothesis that iPS cells in pig can be a useful resource for producing pig model via nuclear transfer. For this study, porcine POU51F, SOX2, c-MYC and KLF4 was expressed by *PiggyBac transposon*, which is inducible expressed by doxycycline. After 24hr transfection, 2ug/mL doxycycline was added in DMEM/F12 culture media contained 15% FBS, 10ng/ml bFGF and transfected cells were cultured for 2 weeks more. From about 10 days after, outgrowing colonies were formed, picked, and cultured on mytomycin C treated mouse feeder cells. They are routinely single cell passaged 1:4~6 at every 3-4 day by enzyme. We verified the colony formation from single cell culture and maintained the colonies up to passage (> 30^{th}). Furthermore, the cells were positive for AP, Oct4, Sox2 and cdy1. To assess the potential for using the cells as the donor for nuclear transfer, single iPS-like cell were reprogrammed into enucleated oocyte and developed into pre-implantational stage. Out of 473 cloned embryos, 207 embryos were reached into 2 cell stage and 31 blastocysts were generated. In conclusion, the data demonstrated that porcine iPS-like cells derived from fetal fibroblasts can be propagated without senescence as single cell culture with self-renewal feature and expression of stem cell markers were successfully reprogrammed into blastocysts stage. This study was supported by grants from, NRF (#550-20110026), Biogreen (PJ0090962012), IPET (#109023-05-1-CG000) and BK21 program.

T-2188

DERIVATION OF AUTOLOGOUS INDUCED PLURIPOTENT STEM CELLS FROM NUCLEAR TRANSFER-RECONSTRUCTED EMBRYOS.

Olivia Eilers Smith[1], Jacinthe Therrien[1], Bruce D. Murphy[1], Lawrence C. Smith[2]
[1]*Centre de Recherche en Reproduction Animale, Université de Montréal, Saint-Hyacinthe, QC, Canada,* [2]*Dept. Reprodução Animal, FCAV-UNESP, Jaboticabal, Brazil*

Equine musculoskeletal tissue shows slow and inefficient recovering capacity following injury. Mesenchymal stem cells (MSC) are currently the focus of new therapy being developed for athletic injuries. However, due to the limited proliferative and differentiation capability of MSC, embryonic stem (ES) cells are often preferred for their pluripotency and unlimited proliferation. Nonetheless, equine ES cells undergo differentiation and slow proliferation after a few passages. Though stable induced pluripotent stem (iPS) cells have been obtained by genetically reprogramming equine somatic cells, embryonic and fetal cells are often preferred due to their less differentiated nature and consequent requirement for shorter exposure to reprogramming factors before the appearance of ES-like colonies. From a clinical perspective, fetal-derived iPS cells are not ideal for in vivo therapy because of their allogeneic nature. Since clones are genetically compatible, we hypothesized autologous iPS lines could be efficiently obtained from fetuses and embryos derived by somatic nuclear transfer. Equine early stage embryos (day 7-9 blastocysts) were produced by somatic cell nuclear transfer (SCNT) and either transferred to recipient mares to derive day 40 fibroblasts or cultured in vitro to obtain ES-like outgrowths for experiments. After trypsin digestion (TrypLE; Invitrogen), fetal fibroblasts (FF-iPS) and some SCNT ES-like cells (ES-iPS) were transfected using a piggyBac (PB) transposon-based technique to deliver transgenes containing the reprogramming factors Oct4, Sox2, Klf4 and c-Myc. Cells were cultured continuously and passaged weekly on inactivated murine fetal layers in equine iPS medium (Nagy et al., 2011; Stem Cell Rev 7:693) to examine the timing of ES-like colony formation, morphologic-

al changes and longevity. The expression of endogenous pluripotency markers such as Oct-4, Klf-4, Nanog, Sox-2, Lin28 and SSEA-1 was measured by quantitative RT-PCR and immunocytochemistry (ICC). ES-iPS cells required a third of the time (7days) for successful reprogramming relative to FF-iPS cells from fetal fibroblast (21days) and produced four times the number of iPS colonies (114 vs. 23). In contrast to non-transfected ES-like colonies that showed epithelial morphology within five passages, ES-iPS and FF-iPS lines continued showing rapid proliferation and stable compact ES-like morphology with defined edges for over 15 passages. Quantitative RT-PCR and ICC analysis showed the presence of the pluripotency markers in both types of SCNT-iPS lines. These results indicate that stable autologous equine iPS cell lines with characteristic ES morphology and pluripotency marker expression can be readily obtained from SCNT derived ES-like cells. These results generate great expectation for future regenerative therapy of diseases that are currently impossible to cure.

T-2191

WHOLE TRANSCRIPTOME PROFILING OF FTD-PSP PATIENT DERIVED IPS DIFFERENTIATED NEURONS

Israel A. Hernandez Moreno, Vesna Cerovac, Mary L. Arcila, Matthew Lalli, Hongjun Zhou, Bruce L. Miller, Fen B. Gao, Yadong Huang, Kenneth S. Kosik
Neuroscience Research Institute, University of California Santa Barbara, Santa Barbara, CA, United States

Neurodegenerative diseases are a group of heterogeneous diseases with diverse symptoms that progressively impair CNS function. Frontotemporal dementia and progressive supanuclear palsy (FTD and PSP) share the histological presence of hyperphosphorylated tau intracellular inclusions known as neurofibrillary tangles. Induced pluripotent stem cells (iPSC) were obtained by four factor reprogramming of skin fibroblasts from a patient diagnosed with FTD/PSP who harbors an A152T polymorphism in tau which is known to increase the risk for the disease. Three iPSC clones of this patient were differentiated to human neurons (hiPS-N). Skin fibroblasts from a clinically healthy A152T carrier beyond the expected age of FTD onset, and two control individuals were reprogrammed to iPSC and differentiated to neurons. These hiPS-Ns were matured in vitro for five weeks, and expressed neuronal markers such as beta-III-tubulin, MAP2, Synapsin I, Tau and PSD 95. hips-Ns from A152T carriers lacked axonal polarity, evidenced by somato-dendritic presence of axonal proteins such as Synapsin I, Tau and the axonal neurofilament antigen SMI312. The severity of the axonopathy correlated with clinical evaluations of the patients. We deep sequenced the transcriptome of the hiPS-Ns matured in vitro. Transcriptomes were obtained from total RNA and polyA selected RNA on the SOLiD platform. Mapping sequences were analyzed for coverage of consensus coding sequences (CCDS) to determine expression levels. Transcriptomes from mouse forebrain and HEK293T cells were used for comparison. Differentially expressed (DE) genes were determined by comparing transcriptomes on the series of hiPS-Ns, and thereby establishing a set of candidates linked to the pathology of FTD/PSP.

T-2192

GENERATION OF MELANOMA ANTIGEN-SPECIFIC T CELLS FROM IPSCS DERIVED FROM MATURE CD8+ T CELLS

Raul Vizcardo[1], Kyoko Masuda[2], Daisuke Yamada[1], Tomokatsu Ikawa[2], Kanako Shimizu[3], Shin-Ichiro Fujii[4], Haruhiko Koseki[1], Hiroshi Kawamoto[2]
[A]Laboratory for Developmental Genetics, [B]Laboratory for Lymphocyte Development, [C]Research Unit for Therapeutic Model, [D]Research Unit for Cellular Immunotherapy, [1]RIKEN Yokohama, RCAI, Yokohama, Japan

Antigen-specific T cells represent a potential therapeutic avenue for a variety of conditions, but current approaches for generating such cells for therapeutic purposes are limited. In this study, we established iPSCs from mature cytotoxic T cells specific for the melanoma epitope MART-1. When cocultured with OP9/DLL1 cells, these iPSCs efficiently generated TCRb+CD4+CD8+ double positive cells (DP) expressing a T cell receptor (TCR) specific for the MART-1 epitope. Stimulation of these DP cells with anti-CD3 antibody generated a large number of CD8+ T cells, and more than 90% of the resulting cells were specific for the original MART-1 epitope. Stimulation of the CD8+ T cells with MART-1 antigen-presenting cells led to the secretion of IFNg, demonstrating their specific reactivity. The

present study therefore illustrates an approach for cloning and expanding functional antigen-specific CD8+ T cells that might be applicable in cell-based therapy of cancer.

T-2192

HUMAN INDUCED PLURIPOTENT STEM CELLS FROM THE HUMAN URINARY TRACT CELLS ARE ABLE TO REGENERATE BLADDER CELLS

Mohammad Moad[1], Deepali Pal[1], Anastasia C. Hepburn[1], Stuart Williamson[1], Laura Wilson[1], Majlinda Lako[2], Lyle Armstrong[2], Simon W. Hayward[3], Omar Franco[3], Justin M. Cates[3], Stefan Przyborski[4], Craig N. Robson[1], Rakesh Heer[1]

[1]Northern Institute for Cancer Research, Newcastle upon Tyne, United Kingdom, [2]Institute of Genetic Medicine, Newcastle upon Tyne, United Kingdom, [3]Vanderbilt University Medical Center, Nashville, TN, United States, [4]School of Biological and Biomedical Science, Durham University, United Kingdom

Background: Direct reprogramming of human somatic cells to pluripotent embryonic stem cell (ESC)-like cells, termed induced pluripotent stem cells (iPSCs), can be achieved by expression of defined transcription factors. The potential use of iPSCs derived from the urinary tract provides a substantial opportunity in developing new disease models, drug screening and tissue engineering. We aimed to generate, for the first time, human induced pluripotent stem cells derived from the human urinary tract (hUT-iPSC) and to assess capacity for directed differentiation into bladder lineages.
Methods: Human primary benign bladder and ureters cells were transduced with OCT4, SOX2, KLF4 and C-MYC to generate hUT-iPSCs. Generated cells were characterised using RT-PCR and immunofluorescence. Differentiation capacity was evaluated by embryoid body formation *in vitro* and teratoma assay *in vivo*. hUT-iPSCs were directed to differentiate into bladder cells using conditioned medium (CM) from cultures of primary urothelium or urothelium associated stromal cells for 14 days. Established co-culture based directed differentiation into bladder cells was assessed in comparison with classical skin-derived iPSCs.
Results: We demonstrated successful reprogramming of adult human urinary tract cells from both bladder and ureter into hUT-iPSCs. Specifically, hUT-iPSCs were very similar to human ESC with respect to morphology and gene expression. They were positive for many pluripotency markers at both RNA and protein level including SSEA4, TRA-1-81, TRA-1-60, OCT4, and NANOG and in comparable levels to human ESC. Most of the clones showed efficient transgene silencing and maintained a normal diploid karyotype. Furthermore, hUT-iPSCs showed functional pluripotency by the generation of endodermal, ectodermal and mesodermal lineages. Differentiation into bladder lineages was demonstrated by expression of the urothelial-specific markers uroplakin (UPIb, UPII, UPIIIa, and UPIIIb), claudin (CLD1 and CLD5) and cytokeratin (CK7); and stromal smooth muscle markers a-SMA, calponin, and desmin. hUT-iPSCs were shown to be more efficient than skin-derived iPSC in generating bladder differentiation, underlining the importance of the origin of the parent cell for reprogramming.
Conclusions: We demonstrated that the induction of human urinary tract into iPSCs is possible, offering a new exciting opportunity for tissue engineering and for the study of bladder disease.

T-2194

USING INDUCED PLURIPOTENT STEM CELLS TO INVESTIGATE THE NEUROBIOLOGY OF PRADER-WILLI SYNDROME

Lisa C. Burnett[1], Carlos R. Sulsona[3], Liheng Wang[1], Daniel J. Driscoll[3], Dieter Egli[4], Rudolph L. Leibel[2]

[A]Nutrition & Metabolic Biology, [B]Pediatrics, [1]Columbia University, New York, NY, United States, [2]Pediatrics, University of Florida, Gainesville, FL, United States, [3]Pediatrics, New York Stem Cell Foundation, Columbia University, New York, NY, United States

Prader-Willi syndrome (PWS) is the most commonly diagnosed syndromic obesity. Seminal phenotypes include feeding difficulties in infancy, infantile hypotonia, and beginning in childhood, hyperphagic obesity, diminished mental capacity, and behavioral problems. PWS is typically caused (~70%) by a large 5-6 Mb deletion on the pa-

ternal 15q11.2-11.3 but can also be caused by uniparental maternal isodisomy, imprinting defects, and microdeletions. Because the maternal chromosome is imprinted at 15q11.2-11.3, PWS patients harboring a deletion, maternal isodisomy, or imprinting defect are functionally null for twelve protein-coding and non-coding genes. Efforts to understand central nervous system defects in PWS have been hindered by failure of PWS mouse models to fully recapitulate the human phenotypes, and limited access to relevant human biological material. The reprogramming of human fibroblasts to induced pluripotent stem cells enables novel approaches to PWS, specifically, the opportunity to identify the genes and molecular mechanisms underlying the nervous system phenotypes. PWS fibroblast lines were obtained from patients with 5-6 Mb deletions on 15q11.2-11.3 (comprising ~70% of PWS genotypes). Fibroblasts were reprogrammed to iPS cell (iPSC) lines. As iPS-related reprogramming is associated with erasure of some epigenetic marks, PWS iPS cells were investigated to determine if maternal DNA methylation on 15q11.2-11.3 remained intact. Genes within the PWS region, including SNURF and NDN, showed persistence of DNA methylation patterns after iPS reprogramming, while SNRPN and SNORD116 remained unexpressed. These data indicate that PWS iPS cell lines retained maternal methylation status in the PWS region. Because PWS imprinting and gene expression patterns were preserved after reprogramming, it may be anticipated that PWS iPS-derived neuronal-like cells would display characteristic differences in transcripts related to energy intake. PWS and control iPSC were differentiated into neuron-like cells. PWS neuron-like cells express neuronal markers including MAP2, TUJ1, Nefl, and NES. By qRT-PCR, SNRPN and SNORD116 remained silenced after differentiation into neuron-like cells. Quantitative and qualitative aspects of the transcriptional repertoire of these cells are under investigation.

T-2195

STAT3 EPIGENETICALLY CONTROLS PLURIPOTENCY ESTABLISHMENT DURING MOUSE IPSC INDUCTION

Yong Tang, Yan Luo, Zongliang Jiang, Xueming Zhao, Xiuchun C. Tian
University of Conencticut, Storrs, CT, United States

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) hold great promises in regenerative medicine. Leukemia inhibitory factor (LIF) activates the Janus kinase/signal transducer and activator of transcription 3 (Jak/Stat3) pathway to maintain pluripotency and self-renewal of naïve state mouse ESCs and iPSCs, however the downstream mechanism(s) of Jak/Stat3 remains elusive. We have recently reported that Jak/Stat3 signal is essential for complete pluripotency establishment via epigenetic mechanisms. Specifically, Stat3 enable complete activation of pluripotent genes by inhibiting the expression of DNA methyltransferase 1 and class I Histone deacetylases during reprogramming. Previously the only reported epigenetic role of Stat3 was that it promotes the expression of polycomb repressive complex 2 (PRC2) key component embryonic ectoderm development gene (Eed) for the maintenance of mouse ESCs, by maintaining Histone 3 lysine 27 trimethylation and the subsequent silencing of linage commitment gene expression. Here we report new evidence of Stat3's role in mouse somatic cell reprogramming by promoting the expression of Eed in reprogrammed cells. In addition, Stat3 also inhibits the expression of the core component of the somatic cell chromatin remodeler SWI/SNF complex — Brm. These finding extended our previous discovery of Stat3's regulatory role in DNA demethylation and histone acetylation during reprogramming, and highlighted the role of Stat3 for the pluripotency establishment from somatic cells, by regulating histone methylation and chromatin structure to suppress the expression of linage commitment genes. Our results also shed new lights on the mechanism of Stat3's role in pluripotency maintenance of naïve ESCs.

T-2196

TRANSPLANTATION OF MOUSE IPS CELL-DERIVED NEURAL PROGENITORS INCREASES NEUROGENESIS AND ANGIOGENESIS IN A FOCAL ISCHEMIC STROKE MODEL

Monica Chau, Shan Ping Yu, Ling Wei
Emory University School of Medicine, Atlanta, GA, United States

Ischemic stroke is a leading cause of death and disability, yet there are limited treatments available for it. Induced pluripotent stem (iPS) cells derived through the upregulation of pluripotent genes (such as (Oct3/4, Sox2, Klf4, and

c-Myc) in adult somatic cells have provided a new source of stem cells that circumvents the ethical concerns of using embryonic stem (ES) cells. These pluripotent cells can be differentiated into neurons. The transplantation potential and therapeutic benefits of iPS cells however, is relatively unknown. We hypothesized that iPS cell-derived neural progenitors have a unique potential for stroke therapy by providing cell replacement, beneficial growth factors to the stroke area, and increasing endogenous migration of neural progenitors in an ischemic stroke adult mouse model.
Pluripotent iPS cells were differentiated into neural progenitors with an 8-day retinoic acid protocol and plated for in vitro differentiation and analysis. iPS cell-derived neurons were whole-cell patch-clamped in vitro to characterize its neuronal activity (i.e. presence of action potentials). Trophic factor expression of these cells was analyzed through immunocytochemistry and Western blot. 2-month old C56BL/6 adult mice received focal ischemic stroke in the whisker barrel cortex and the forepaw somatosensory area by a permanent occlusion of the middle cerebral artery and a 7-minute temporary occlusion of the common carotid artery. 400,000 iPS cell-derived neural progenitors were transplanted into the stroke penumbra 7 days after stroke induction. 7 days was chosen as a transplantation time point to avoid the cytotoxic milieu from the acute phase of cell death. The mice received daily BrdU injections starting on the day of transplantation and were sacrificed 7d after transplantation/14d after stroke for immunohistochemistry (anti-NeuN, anti-Collagen IV, anti-BrdU). Behavioral tests were performed to evaluate sensorimotor functional recovery.
Pluripotent mouse iPS cells were successfully differentiated into neurons expressing neuronal markers, NeuN, neurofilament, and MAP-2 in vitro (87.5% of cells in vitro differentiated into NeuN-positive cells). iPS cell-derived neural progenitors and undifferentiated iPS cells expressed the trophic factors, VEGF, EPO, SDF-1, FGF, and GDNF which are known to play roles in neurogenesis and/or angiogenesis. iPS cell-derived neurons fired action potentials, had an inward sodium current and outward potassium current in vitro. Addition of tetrodotoxin (TTX) abolished the sodium current. Stroke mice that received iPS cell-derived neural progenitor transplantation had more BrdU-positive cells, BrdU/NeuN, and BrdU/Collagen IV double-labeled cells compared to stroke only mice. Cell transplantation animals showed improved sensorimotor function via the adhesive removal test to test forepaw sensorimotor abilities.
The increased number of new neurons and vessels in the ischemic brain suggests increased neurogenesis and vascular remodeling with iPS cell-derived progenitor transplantation. Transplantation of these cells act as a vehicle for supplemental trophic factors to the stroke area. Trophic factors have shown to increase endogenous progenitor migration to the infarct, cell survival, and endogenous neurogenesis and angiogenesis. In this study, we demonstrate that iPS cell transplantation increases regenerative activities in the stroke brain and is a potential therapy for ischemic stroke.

T-2197

SURVIVAL AND INTEGRATION OF PLURIPOTENT STEM CELL-DERIVED NEURAL PROGENITOR GRAFTS AFTER FOCAL CEREBRAL ISCHEMIA IN ADULT RATS

Danielle K. Lewis, Aaron C. Ajlen, Yu Liu, Helen Zhang, Jack M. Parent
Neurology, University of Michigan Health System, Ann Arbor, MI, United States

Stem cell transplantation offers a promising regenerative therapy for stroke and other brain insults. However, widespread clinical application requires a better understanding of neural stem cell-host interactions. For example, the origin and differentiation status of the stem cells as well as the local microenvironment in the brain following injury will likely influence stem cell proliferation, differentiation, migration and integration. In this study, induced pluripotent stem cell (iPSC)-derived neural progenitors (100,000 cells) were transfected with a retrovirus containing the neuron-specific human synapsin-1 promoter (SYN) driving a synaptic reporter (synaptophysin-yellow fluorescent protein; SYP-YFP) prior to grafting. SYN-SYP-YFP-infected cells were injected into the striatum of young adult male rats following transient middle cerebral artery occlusion (tMCAO). Induced pluripotent stem cell-derived neural progenitor cell suspension cultures were grown in a neuron-specific medium for 1 week prior to grafting. Brains were collected at 48-hours, 28- and 56-days post grafting and immunohistochemistry was performed to examine proliferation, survival, migration and phenotypes of the grafted cells. Brains were co-labeled for the human-specific

cell marker, human nuclear protein (HNP), and either Ki67, a marker for cell proliferation; doublecortin, a marker for neuroblasts; or glial fibrillary acidic protein (GFAP), an astrocytic marker. iPSCs (HNP+) were observed in the striatum at 48-hours and cells migrated both rostrally and caudally by 28-days. At 28-days post grafting a large proportion (63%) of the HNP+ cells co-labeled for the neuroblast marker doublecortin while few co-expressed Ki67 (11%) or GFAP (0.08%), suggesting that most grafted cells differentiated into post-mitotic neurons. Interestingly, co-labeling for YFP and the neuronal marker, MAP2 also suggested that grafted cells formed synapses with host cells. YFP+ axons and synaptic terminals were observed in the globus pallidus, a projection area of the striatum. These findings suggest that iPSC-derived neural progenitor transplants survive, migrate and differentiate into neurons that form long-distance synaptic projections following stroke. Behavioral analysis to examine the functional significance of iPSC grafting after stroke and the fate of iPSC-derived neural progenitors at the 56-day time point are in progress.
Supported by: NS065450 to JMP

T-2198

INDUCED PLURIPOTENT STEM CELLS ACQUIRE MUTATIONS DURING SOMATIC CELL STATE PRIOR TO THE ACQUISITION OF PLURIPOTENCY

Rui Liu[1], Athanasia D. Panopoulos[2], Athurva Gore[1], Ho-Lim Fung[1], Juan Carlos Izpisua Belmonte[2], Kun Zhang[1]
[1]Bioengineering, UC San Diego, La Jolla, CA, United States, [2]Gene Expression Laboratory, Salk Institute for Biological Studies, La Jolla, CA, United States

Somatic point mutations and copy number changes have been ubiquitously identified in human and mouse induced pluripotent stem cells (iPSCs) regardless of the method of reprogramming or the somatic cell type of the progenitor. Certain fractions of point mutations were identified as pre-existing in the starting somatic cell populations. However, the origin of such mutations and the underlying mechanism still remain elusive. In this study, we sought to probe this issue by whole genome sequencing iPSC lines at various stages of reprogramming.
We reprogrammed a human fibroblast line containing an Oct4-driven GFP reporter. We carefully dissected micro-colonies during reprogramming immediately after the cells acquired pluripotent characteristics based on GFP expression. One quarter of each colony, containing roughly 250 cells, was further expanded to an iPSC line for standard characterization. The remaining cells in each earlier colony, additional cells from each line at later passages during expansion, and the progenitor fibroblasts were whole-genome sequenced at ~40X coverage. We generated data from three early-stage iPSC colonies, plus cells at 1-2 later stages for each colony, for a total of nine whole genome sequencing data sets.
We found over one thousand somatic mutations in each line genome-wide. Roughly 2/3 of these mutations were shared among different colonies, while the rest appeared to be line specific. We did not find evidence that the majority of mutations occurred after pluripotency was established in the iPSC micro-colonies. We next searched for epigenetic signatures that might potentially be enriched at or near the mutation sites. Our analysis identified distinct epigenetic patterns for shared and line specific mutations. A clear enrichment of DNase I sensitivity in fibroblasts but not embryonic stem cells was found for shared mutations, which is consistent to the notion that mutations shared by multiple independent iPSC lines pre-existed in the fibroblast progenitors. For lineage-specific mutations, the H3K4me3 enrichments (a mark for active promoters) were much stronger for fibroblasts and a number of other somatic cell types than for hESCs. Such fibroblast-specific enrichment of epigenetic signatures suggests that mutagenesis most likely occurred in the somatic cell state prior to the transition to pluripotency. Finally, for the lineage-specific mutations, we observed a highly localized enrichment of H3K9me3, which was recently shown to be the strongest epigenetic predictor of cancer mutations and a unique epigenetic signature for *in vitro* cell culture. This raises the possibility that *in vitro* culture and expansion of somatic cells is primarily responsible for the mutational load observed in iPSCs.

T-2201

THE HUMAN STEM CELL TECHNIQUES COURSE AT THE SCRIPPS RESEARCH INSTITUTE CENTER FOR REGENERATIVE MEDICINE

John P. Schell, Yu-Chieh Wang, Eyitayo S. Fakunle, Heather Schultheiz, Ron Coleman, Andres Bratt-Leal, Ileana Slavin, Victoria Glenn, Ha T. Tran, Kristopher Nazor, Michael Boland, Suzanne E. Peterson, Jeanne F. Loring
The Scripps Research Institute, San Diego, CA, United States

Advances in regenerative medicine have increased the demand for highly trained individuals to fill the demands of academic and industrial research and development. The Scripps Research Institute Center for Regenerative Medicine has developed two types of training courses to train entry level and professional researchers in human pluripotent stem cell technologies. Our entry-level courses are for the Bridges to Stem Cell Research program sponsored by the California Institute for Regenerative Medicine (CIRM). The course for professionals is generally for local graduate students, technicians, postdoctoral researchers and heads of labs, but we also accommodate other US and international researchers and hold specialized courses for groups on request. We offer about 4 to 6 courses per year, depending on demand and availability of the lab, which is also used for active research projects. The classes typically last 5-12 days, with one to two hours of lectures and several hours of hands-on lab work each day. The laboratory training includes methods for generation of human iPSCs, care of human pluripotent stem cells, and methods for characterizing pluripotency and differentiation. The lectures cover not only general principles about stem cells, but also aspects of the techniques we will be using in the course, and we always include a lecture on ethics. All students are provided with the manual “Human Stem Cell Manual: A Laboratory Guide” (now in 2nd edition) written by Drs. Loring and Peterson, and a folder containing general stem cell information, lecture handouts, protocols for each technique used in the course and seminal papers in stem cell research. Over the last 5 years, we have trained more than 200 students; the majority use the courses as a starting point for involvement in stem cell research, either as interns, graduate students, postdocs, or leaders of their own labs.

Reprogramming

T-2203

AN EPIGENETIC COMPONENT OF HEMATOPOIETIC STEM CELL AGING AMENABLE TO REPROGRAMMING INTO A YOUNG STATE

Martin Wahlestedt[1], Gudmundur L. Norddahl[1], Gerd Sten[1], Amol Ugale[1], Mary-Ann Micha Frisk[2], Ragnar Mattson[2], Tomas Deierborg[3], Mikael Sigvardsson[4], David Bryder[1]
[A]Immunology, [B]Transgenic Core Facility, [C]Neuronal Survival Unit, [1]Experimental Medical Science, Lund, Sweden, [2]Institution of Clinical and Experimental Medicine, Linköping, Sweden

Aging associates with an altered function of somatic stem cells, which in turn has been hypothesized to underlie several aspects of organismal aging. In the blood system, increasing age is associated with an altered lineage distribution of mature cells and an expansion of the hematopoietic stem cell (HSC) pool, although the latter is accompanied with several functional shortcomings. Moreover, the incidence of anemias and myelogenous leukemias is increased in the elderly. Since HSCs maintain the hematopoietic system, modulating their function upon aging may prove a promising route to restore compromised hematopoietic function.
While misappropriate genomic maintenance has been proposed to accompany aging somatic stem cells, models of compromised DNA repair have thus far failed to mirror all the functional changes of stem cell aging. The exact mechanisms by which physiological age alters HSCs is currently not known. However, it is well established that aging HSCs associate with an altered transcriptome compared to their young counterparts. We could verify these findings and next investigated the stability of these gene expression perturbances. To this end, we initially transplanted young and aged lineage depleted bone marrow into lethally irradiated recipient mice. 4 months later, we isolated highly purified HSCs from these animals and performed genome-wide transcription analyses. When intersecting the age-associated differentially expressed genes with those of the “steady-state” young and aged HSCs we found a strong correlation. Thus, it seems as the transcriptional alterations occurring in aging HSCs are of a highly stable nature, and therefore likely associate with the functional shortcomings associated with aging HSCs.
Finally, we wondered whether a normalization of the deregulated aging transcriptome of HSCs could have functional benefits. To achieve this, we used cellular reprogramming of hematopoietic progenitor cells into induced

pluripotent stem cells (iPS), followed by re-differentiation of iPS cells into HSCs *in vivo*. We found that the HSCs derived from iPS cells with an aged somatic origin were functionally indistinguishable from young HSCs in terms of differentiation and reconstitution capacity. While, we could find a slight reduction in the telomere length of aged compared to young HSCs (11%), iPS derived HSCs harbored telomeres almost twice the length of normal HSCs regardless of donor age. Our studies establish that a reversible epigenetic component underlie physiological somatic stem cell aging, and suggests that donor age might be dispensable for successful future iPS-based cell replacement therapies.

T-2204

CAPTURING DYNAMIC REGULATORS OF NUCLEAR REPROGRAMMING IN HETEROKARYONS

Helen M. Blau, Jennifer J. Brady, Wing H. Wong, Matthew Decker, David Burns
Stanford Univ Sch of Medicine Baxter Lab for Stem Cell Biology, Stanford, CA, United States

We have developed an experimental system that offers a means to explore the regulatory networks and the logic underlying nuclear reprogramming to diverse cell fates. Using cell fusion to form stable non-dividing multi-nucleated heterokaryons, our laboratory previously showed that silent human genes could be activated in a range of differentiated cell types. These experiments ran counter to the prevailing dogma that a cell's destiny was "terminal" and irreversible and provided the first evidence that human cells of all three lineages could be induced to express muscle genes that they normally would never express. The stoichiometry of regulators was key for driving this process.
The discovery of induced pluripotent cells (iPS) heralded a new context for the principles of cellular plasticity derived from heterokaryons. Indeed, the low efficiency of iPS generation (0.01%) remains a conundrum. We reasoned that there is a lack of mechanistic insights that could be provided by the heterokaryon system. By skewing the ratio of mouse embryonic stem (ES) cells fused to human fibroblasts, we ensured a shift in the balance of regulators and reprogramming toward a pluripotent state. Heterokaryons proved advantageous, as the onset of reprogramming is synchronous, rapid, and efficient (70%). By exploiting and refining novel molecular methods and algorithms we could examine the panoply of genes expressed, even transiently, using transcriptome-wide bi-species deep sequencing. Importantly, no transcripts are lost due to species homology. Our heterokaryon studies established a transient role in iPS generation for a secreted factor that can substitute for one of the four transcription factors, the oncogene c-Myc, and revealed that an enzyme implicated in active DNA demethylation is crucial to the onset of reprogramming toward pluripotency in both heterokaryon and iPS systems. Global molecular analyses in heterokaryons offer unique insights into directed reprogramming by providing molecular time-lapse snapshots of discrete steps in pathways of dedifferentiation and redifferentiation.

T-2205

HIERARCHICAL MECHANISMS IN TRANSCRIPTION FACTOR MEDIATED REPROGRAMMING OF FIBROBLASTS TO NEURONS

Qian Yi Lee[1], Orly Wapinski[1], Thomas Vierbuchen[2], Kun Qu[1], Ben Martynoga[3], Diogo Castro[3], François Guillemot[3], Howard Chang[1], Marius Wernig[1]
[1]Stanford University, STANFORD, CA, United States, [2]Harvard University, Cambridge, MA, United States, [3]MRC National Institute for Medical Research, London, United Kingdom

Direct lineage reprogramming is a potentially powerful approach in studying mechanisms that underlie cell fate determination, as well as for disease modeling and drug discovery. Previously, we were able to successfully reprogram fibroblasts to neurons (also known as induced neurons or iNs) with the transcription factors Brn2, Ascl1 and Myt1l (BAM factors). We have also shown that Ascl1 is sufficient to initiate the reprogramming process to form immature neurons, and that the addition of Brn2 and Myt1l produce functional neurons with more complex morphologies. Here, we show that there is a hierarchical mechanism involved in reprogramming process. Ascl1 acts as a pioneering factor by immediately binding its cognate genomic sites and opening up the local chromatin to make

it competent for other transcription factors to bind. In contrast, Brn2 and Myt1l do not access chromatin competently on their own early on in reprogramming. Instead, a large fraction of Brn2 appears to be recruited to Ascl1 binding sites genome-wide. These findings correlate strongly with the rapid transcriptional changes we observe as early as 48 hours after induction of the BAM factors in MEFs (mouse embryonic fibroblasts). Induction of individual factors in MEFs shows that Ascl1 alone contributes to the majority of the genes up-regulated 48 hours after BAM induction, while Brn2 and Myt1l show more subtle transcriptional changes. This suggests that Ascl1 may be the strongest driver of the activation of the neuronal transcriptional program, while Brn2 and Myt1l may play a more significant role during the later stages of neuronal maturation. Ascl1's pioneering activity at its cognate genomic sites during the early stages of the reprogramming may also explain the high efficiency of iN formation compared to other systems such as induced pluripotent stem cells. All together, these data suggest that pioneer factor activity is critical for the initiation of transcriptional reprogramming in a non-native chromatin context.

T-2206

CELL CYCLE AND P53 GATE THE DIRECT CONVERSION OF HUMAN FIBROBLASTS TO DOPAMINERGIC NEURONS

Houbo Jiang[1], Zhimin Xu[2], Ping Zhong[1], Xiaomin Wang[3], Shengdi Chen[2], Zhen Yan[1], **Jian Feng**[1]
[1]Department of Physiology and Biophysics, State Univ of New York at Buffalo, Buffalo, NY, United States, [2]Department of Neurology & Institute of Neurology, Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China, [3]Department of Neurobiology, Capital Medical University, Beijing, China

The direct conversion of fibroblasts to induced dopaminergic (iDA) neurons and other cell types has significant impact on many practical applications and fundamental understanding of cell biology. The low efficiency of these relatively fast conversions, which generally manifest themselves within days, suggests that conditions additional to the requisite transcription factors must be met to enable highly efficient cellular reprogramming. Here, we show that reduction in p53 expression significantly improved the efficiency of generating iDA neurons from human fibroblasts using Ascl1, Nurr1, Lmx1a and miR124. In addition, cell cycle arrest at the G1 phase by serum withdrawal greatly enhanced the conversion. Furthermore, a combination of small-molecule compounds and neurotrophic factors that facilitate the differentiation, survival and maturation of dopaminergic (DA) neurons significantly boosted the conversion efficiency so that 93% of the cells at day 9 were Tuj1+ and 59% were TH+. The iDA neurons expressed markers for midbrain DA neurons, had Ca2+-dependent DA release and selective DA uptake, and exhibited spontaneous action potentials and spontaneous excitatory postsynaptic currents. The results suggest that cell cycle arrest at G1 readies mitotic cells such as fibroblasts for conversion to postmitotic cells such as neurons. Reduction in p53 level appears to remove a key barrier of cellular reprogramming, as is found in the derivation of iPS cells. The optimal combination of survival factors enables the manifestation of the efficient conversion process. These three parameters (cell cycle, p53 and extracellular environment) may be important to cellular reprogramming in general.

T-2207

INDUCED NEURONAL CELLS FOR MODELING A SYNAPTIC AUTISM-ASSOCIATED PHENOTYPE PRODUCED BY A NEUROLIGIN MUTATION

Soham Chanda[1], Samuele Marro[2], Jason Aoto[3], Marius Wernig[2], Thomas C. Sudhof[3]
[A]ISCBRM, SINTN, [B]ISCBRM, [C]SINTN, [1]Stanford University, Stanford, CA, United States

Recent studies suggest that analysis of induced neuronal (iN) cells reprogrammed from non-neuronal lineages provides a powerful opportunity to examine neuropsychiatric diseases. However, the validity of this approach using iN cells to understand disease specific traits has never been demonstrated. Here, we directly compare the phenotypes of primary neurons and of iN cells that were derived from the same mutant mice carrying the autism-associated R704C substitution in neuroligin-3. We show that neuroligin-3 R704C-mutant iN cells exhibit a selective, approximately 50% decrease in AMPA-type glutamate receptor mediated synaptic transmission without changes in NMDA-type glutamate or in GABA-receptor mediated synaptic responses. We observed the identical synaptic de-

fect in primary neurons from the same mouse strain. Thus, the iN cells replicated the cellular phenotype of primary neurons induced by a specific mutation. These data constitute an important first proof-of-concept demonstrating that iN cells can be used for cellular disease modeling.

T-2208

IN VIVO REPROGRAMMING...AND BEYOND

LLuc Mosteiro[1], Maria Abad[1], Cristina Pantoja[1], Sagrario Ortega[1], Marta Cañamero[1], Orlando Domínguez[1], Teresa Rayón[2], Miguel Manzanares[2], Manuel Serrano[1]
[1]CNIO, Madrid, Spain, [2]CNIC, Madrid, Spain

Despite great progress in recent years, and the increasing enthusiasm in the field of reprogramming, little is known regarding the hypothetical feasibility or existence of the reprogramming processes in vivo. To shed some light on this issue, we have generated a mouse model in which the four Yamanaka factors are ubiquitously expressed. The expression of the factors is induced upon doxycycline treatment, leading to a synchronised and stoichiometric activation of the reprogramming factors in the adult organism. The reprogrammable mouse has enabled us to study the effects of the expression of the Yamanaka factors in vivo.

T-2211

DIRECT CONVERSION OF FIBROBLASTS INTO DIFFERENT NEURONAL SUBTYPES

Yi Han Ng, Nan Yang, Soham Chanda, Marius Wernig
Stanford Univ, Stanford, CA, United States

Direct reprogramming is a relatively recent development in the field of reprogramming. This fast and efficient strategy converts one cell type directly into another cell type. Besides opening up potential new sources for cell replacement therapy, direct reprogramming also circumvents many of the pitfalls associated with obtaining differentiated cells *in vitro* from embryonic stem cells. Previously, our lab has demonstrated direct reprogramming of mouse and human fibroblasts into functional induced neuronal (iN) cells using defined transcription factors *Brn2*, *Ascl1*, *Myt1L* and *NeuroD1*. Here, we aim to provide some insights and updates on the various subtypes of iN cells that can be generated using combinations of different transcription factors. The various subtypes are generated with defined transcription factors that are crucial in development and cell fate specification. The cells are then characterized based on neuronal markers expression, electrophysiology and *in vivo* assays.

T-2212

INDUCED NEURONS RETAIN SENDAI VECTORS AFTER SUCCESSFUL TRANSDIFFERENTIATION FROM BLOOD

Julio Castaño[1], Amaia Sousa[2], Nerea Vázquez[2], Lorea Zabaleta[1], Xonia Carbajal-Vergara[3], **Rosario Sánchez Pernaute**[2], Alessandra Giorgetti[1]
[A]Hematopoiesis and Blood Disorders Laboratory, [B]Stem Cells and Neurodegenerative Disorders, [C]Cell Reprogramming and Differentiation Platform, [1]Inbiomed Foundation, San Sebastian, Spain

Non integrative vectors based on the Sendai virus, a negative single strand RNA virus, provide a safe and efficient method for reprogramming blood cells to induced pluripotent stem cells (iPS). Induced neurons (iN) have been obtained using different integrative approaches. Indeed we have recently showed that cord blood (CB) CD133+ cells can be directly turn into neurons by overexpression of Sox2. The reprogramming efficiency was improved by the addition of c-Myc. Here we investigated the effect of Sox2 and c-Myc using a non-integrative method based on Sendai virus (SeV, CytoTune®) to derive iN from blood cells. Transduction of CB CD133+ cells was highly efficient at low MOIs. Transduced cells were cultured on astrocytes in the presence of neural induction media, including BMP, TGF-beta and GSK3-beta inhibitors. Under this culture conditions cells displayed a neuroepithelial morpho-

logy within one week. These cells could be greatly expanded (>500 fold in the first 4 passages) and could be freeze down and thawed repeatedly and mantained in culture for several months. Upon differentiation these neuroepithelial cells acquired mature neuronal phenotypes, expressed specific neuronal and synaptic markers and showed electrophysiological properties.
Unexpectedly, we found a persistent expression of the SeV in the iN (p >20), both by immunofluorescence (IF) and by qPCR. By IF SeV particles were found in the cytoplasm and were also prominently localized in neurites and dendritic spines. In contrast, we have not detected any residual expression in our iPS cell lines derived using the CytoTune®kit, after a few passages (p < 8). Furthermore, a temperature-shift treatment (5 days at 39ºC) did not eliminate the SeV and some samples showed an increase in viral RNA (2-3 fold) and protein.
RNA virus can hijack the endogenous RNA protection machinery to survive. Neurons rely on FMRP and some members of the ELAV/Hu family of RNA binding proteins (RBP) to protect mRNAs that need to be transported and expressed in a tightly regulated manner in dendrites. By qPCR we found that HuR and FMRP expression patterns followed that of SeV during passages and temperature-shift treatment of iN. Confocal analyses showed that FMRP but not HuR was colocalized with SeV in some neurites in mature iN. To verify that neuronal specific RBP are involved in this process, we are monitoring SeV levels in SeV-iPS (p < 3) during propagation and upon their neural differentiation over time.
Our findings suggest that neurons may not be able to eliminate the SeV due to specific RNA protection mechanisms. Therefore, further studies are needed to rule out a negative effect of this vector on neuronal function in direct transdifferentiation studies, despite being a first-choice for transgene free iPS production from blood cells.

T-2213

DEFINED OCT4 LEVEL GOVERNS CELL STATE TRANSITIONS OF PLURIPOTENCY ENTRY AND DIFFERENTIATION INTO ALL EMBRYONIC LINEAGES

Aliaksandra Radzisheuskaya[1], Gloryn Le Bin Chia[1], Rodrigo L. dos Santos[1], Thorold W. Theunissen[1], L. Filipe C. Castro[2], Jennifer Nichols[1], Jose C.R. Silva[1]
[1]Wellcome Trust - Medical Research Council Cambridge Stem Cell Institute, University of Cambridge, Cambridge, United Kingdom, [2]CIMAR/CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal

Oct4 is considered a master transcription factor for pluripotent cell self-renewal but its biology remains poorly understood. Here, we investigated the role of Oct4 using the process of induced pluripotency. We found that a defined ES-cell level of Oct4 is a requirement for pluripotency entry. However, once pluripotency is established, Oct4 level can be decreased significantly, up to 7-fold, without loss of self-renewal capacity. Unexpectedly, cells constitutively expressing Oct4 at ES-cell level showed robust ability to differentiate into all three embryonic lineages and germline. In contrast, cells with low Oct4 levels were deficient in their capacity to differentiate, exhibiting high expression of naive pluripotency genes. The restoration of Oct4 expression to ES-cell level rescued the ability of these to restrict naive pluripotency gene expression and to differentiate. In conclusion, we showed that a defined level of Oct4 controls establishment of naive pluripotency as well as commitment to all embryonic lineages.

T-2214

HIGH RESOLUTION ANALYSIS OF CELL STATE PROGRESSION DURING REPROGRAMMING TO ESC-LIKE AND AN ALTERNATIVE INDUCED PLURIPOTENT STATES

Peter D. Tonge, Mira Puri, Samer Hussain, *. International PG consortium, Andras Nagy
Samuel Lunenfeld Research Institute, Toronto, ON, Canada

Somatic cells can be successfully reprogrammed to an induced Pluripotent Stem Cell (iPSC) state that closely resembles Embryonic Stem Cells (ESCs). We have reprogrammed embryonic fibroblasts by transgenic over-expression of Sox2, Oct4, c-Myc and Klf4 and characterized 28 clonal and permanent cell lines unbiased of cell morphology or

gene expression. Global gene expression profiling revealed that the lines segregate in to two distinct clusters. The cluster furthest from ESC-like state (herein referred to as Type-A iPSCs) can be readily maintained in a stable but transgene expression dependent state.
Although many cell lines (including type-A iPSCs) fail to become reprogramming factor independent iPSCs, we have found that they possess the potential to efficiently generate differentiated cell types representative of all three germ layers in vivo, and can be differentiated to terminally differentiated cell types in vitro. Therefore they are considered to be pluripotent.
To study the cell state change between fibroblasts, type-A iPSCs and ESC-like iPSCs, we employed an inducible secondary reprogramming system that efficiently models fibroblast reprogramming to these alternative iPSC states. This system allows for molecular characterization of the entire process of population-based reprogramming starting at a very early stage. We generated a high resolution, genome-wide resource that incorporates transcriptional activity, protein expression and the epigenetic landscape of cells during reprogramming. To enable cross-correlation between the platforms, cell samples were collected in parallel from an experimental run.
For all OMIC platforms, the most dramatic molecular changes lie within the first 48 hours of reprogramming. Subsequent molecular changes continue until they plateau when cells reach the alternative iPS states. RNA sequencing revealed cohorts of genes that exhibit differential transcriptional activity between type-A iPSCs and ESC-like iPS cells. Mapping of the epigenome with respect to histone modification marks (H3K4me3, H3K27me3 and H3K36me3) and CpG methylation enabled us to attribute dynamics of RNA transcription to epigenetic architecture. We found that genes over-expressed in type-A iPSCs typically lack an inhibitory H3K27me3 mark that is found in ESC-like iPSCs. In contrast, ESC-associated genes that are silent in type-A iPS cells are frequently associated with regions of CpG hypermethylation, lack H3K4me3 and possess inhibitory H3K27me3 marks that persist from fibroblast origins.
In summary, this study comprehensively characterizes the diversity of reprogrammed cell states and demonstrates that there are multiple states of pluripotency that do not resemble ESCs. We have mapped the transcriptional and epigenetic changes that fibroblast cells undergo during reprogramming to two pluripotent states - type A iPS state and ESC-like state. Correlating transcription with the epigenetic architecture has allowed us to reveal the mechanisms controlling cell state changes and individual gene expression dynamics.

T-2215

RAPID ERASURE OF DONOR-SPECIFIC EPIGENETIC MEMORY IN BLOOD-DERIVED EPISOMAL HUMAN IPSC AUGMENTS HEMATOPOIETIC POTENCY

Jeffrey S. Huo[1], Tea Soon Park[1], Kurinji Pandiyan[2], C. Conover Talbot Jr.[3], Karan Verma[1], Ludovic Zimmerlin[1], Ann Peters[1], Kejin Hu[4], Igor Slukvin[4], Renee Reijo-Pera[5], Stephen B. Baylin[2], Elias T. Zambidis[1]
[A]Pediatric Oncology & Institute for Cell Engineering, [B]Cancer Biology, Sidney Kimmel Comprehensive Cancer Center, [C]Institute for Basic Biomedical Sciences, [1]Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2]Department of Pathology, University of Wisconsin-Madison, Madison, WI, United States, [3]Stem Cell Biology Program, Stanford University, Palo Alto, CA, United States

Nonviral, non-integrating methods for generating human induced pluripotent stem cells (hiPSC) lines have potential to produce patient-specific stem cells with fewer integration-related safety concerns and potentially greater utility for therapeutic and disease modeling applications. However, substantial variation has been observed in the potency of differentiation of individual hiPSC lines depending on donor cell source and derivation method. Incomplete reprogramming and retention of donor-specific epigenetic memory in early passage hiPSC has been proposed as a factor that hinders differentiation into lineages disparate to the originating donor cell of origin. It has even been proposed that harnessing donor cell-specific memory could enhance or bias differentiation of hiPSC toward the original lineage of the donor cell.
We present findings demonstrating that retention of blood-specific epigenetic memory in hiPSC derived from myeloid progenitors is an obstacle to, not an enhancer of, their hematopoietic differentiation potency. Rapid erasure of blood-specific epigenetic memory through a novel stromal-primed (sp) reprogramming method of myeloid pre-

cursors led to (sp)-myeloid-hiPSC with hematopoietic differentiation potency that was superior to not only blood-derived hiPSC derived via standard methods, but even to hESC. We employed whole genome expression and epigenetic bioinformatics analyses along with differentiation studies of a repertoire of >30 episomal sp-myeloid-hiPSC, standard blood-derived hiPSC, fibroblast-hiPSC, and hESC lines. Superior hematopoietic differentiation potency of sp-myeloid-hiPSC was associated not with retention of blood-specific somatic memory, but with Myc-, Polycomb-, and Core factor-regulated stem cell circuits that were already indistinguishable to hESC at early passages. Furthermore, sp-myeloid-iPSC possessed less retention of donor-cell derived gene expression patterns, had more complete erasure of donor-specific epigenetic memory with fewer reprogramming errors, and were devoid of cancer-associated signatures found in other hiPSC derived via standard methods. CpG DNA Methylation array analysis also revealed that failure to fully reprogram promoter methylation to a pluripotent state accounted for only a minority (~5-10%) of incompletely reprogrammed genes in these hiPSC, thus suggesting additional complex epigenetic events regulating high fidelity reprogramming. These findings refine the notion that donor-specific memory positively skews hiPSC differentiation toward the donor's original lineage. Instead, incomplete erasure of donor gene expression may pose an impediment to pluripotency that can be overcome by efficient derivation methods that produce high-fidelity pluripotent states at early passages. Further technical refinements in non-integrating reprogramming fidelity and efficiency may produce clinically useful hiPSC that are completely indistinguishable molecularly and functionally from bona fide blastocyst-derived hESC.

T-2216

MICRORNA-CONTROLLED CHROMATIN REGULATION REPRESENTS AN ENDOGENOUS ROADBLOCK FOR THE GENERATION OF IPSCS

Nils Pfaff[1], Jan Fiedler[2], Thomas Thum[2], Thomas Moritz[1], Tobias Cantz[3]
[A]REBIRTH RG Reprogramming, [B]IFB-Tx-group Molecular and Translational Therapeutic Strategies, [C]REBIRTH RG Stem Cell Biology, [1]Hannover Medical School, Hannover, Germany

Introduction: MicroRNAs (miRNAs) have repeatedly been demonstrated to play important roles in the generation of induced pluripotent stem cells (iPSCs). To further elucidate the molecular mechanisms underlying transcription factor-mediated reprogramming and to increase the efficiency of this process, we have established a model which allows screening of whole libraries for miRNAs potentially modulating the generation of iPSCs. In this model, murine embryonic fibroblasts (MEFs) from OG2 mice (Oct4-GFP) were transduced with a polycistronic lentiviral construct expressing Oct4, Klf4 and Sox2 and an IRES-coupled dTomato reporter. One day after transduction, miRNAs from a Pre-miR™ Library (Ambion) containing 379 miRNAs were individually transfected into these MEFs. At day 7 to 10, emerging GFP-positive iPSC-like colonies were counted and further analyzed.
Results: Applying this model, we have identified a miRNA family consisting of miR-130b, miR-301b and miR-721, which strongly enhance iPSC generation at least in part through repression of the homeobox transcription factor Meox2, a transcriptional activator of the tumor suppressor genes Cdkn2a and p21. Our analyses also revealed several miRNAs effectively inhibiting iPSC generation upon overexpression, including miR-132 and miR-212. Intriguingly, repression of these miRNAs during iPSC generation led to significantly increased reprogramming efficiencies. This observation was further confirmed by demonstrating that fibroblasts from miR-132/212 knock-out mice displayed significantly increased propensities to undergo reprogramming compared to wild-type cells. miRNA target identification by qRT-PCR, western blot and luciferase assays revealed two crucial epigenetic regulators, the histone acetyl transferase p300 as well as the H3K4 demethylase Jarid1a (KDM5a) to be directly targeted by both miR-132 and miR-212. We further demonstrated that specific siRNA-mediated knockdown of either p300 or Jarid1a recapitulated the miRNA effects and led to a significant decrease in reprogramming events.
Conclusion: Thus, conducting a full library miRNA screen we identified a novel miRNA family strongly enhancing iPSC generation from MEFs and we could associate this to a repression of Meox2. In addition, we found several miRNAs, which strongly restrain iPSC-generation and upon inhibition in turn enhance reprogramming. These miRNAs, at least in part, exert their functions through repression of the epigenetic modulators p300 and Jarid1a, thereby potentially representing an endogenous epigenetic roadblock for the generation of iPSCs. These observa-

tions underline that robust RNAi screening approaches represent a powerful tool to identify novel factors modulating iPSC generation and may substantially add to our understanding of the molecular mechanisms underlying transcription factor-mediated reprogramming.

T-2217

LACTIC ACID BACTERIA CONVERT HUMAN FIBROBLASTS TO MULTIPOTENTIAL CELLS

Kunimasa Ohta, Rie Kawano, Naofumi Ito
Kumamoto University, kumamoto, Japan

Humans are in contact with components of the microflora from birth. Thus, a delicate balance exists in the symbiotic relationship between microorganisms and the human host during metabolic activities. Lactic acid bacteria (LAB) form a group of related, low-GC-content, gram-positive bacteria that are considered to offer a number of probiotic benefits to general health. While the role of LAB in gastrointestinal microecology has been the subject of extensive study, little is known about how commensal prokaryotic organisms directly influence eukaryotic cells.
Here, we demonstrate the generation of multipotential cells from adult human dermal fibroblast cells by incorporating LAB. LAB-incorporated cell clusters are similar to embryoid bodies derived from embryonic stem cells and can differentiate into endodermal, mesodermal, and ectodermal cells in vivo and in vitro. LAB-incorporated cell clusters express a set of genes associated with multipotency, and microarray analysis indicates a remarkable increase of NANOG, a multipotency marker, and a notable decrease in HOX gene expression in LAB-incorporated cells. During the cell culture, the LAB-incorporated cell clusters stop cell division and start to express early senescence markers without cell death. Thus, LAB-incorporated cell clusters have potentially wide-ranging implications for cell generation, reprogramming, and cell-based therapy.

T-2218

LINEAGE CONVERSION OF HUMAN FIBROBLASTS INTO FUNCTIONAL ENDOTHELIAL AND HEMATOPOIETIC CELLS VIA THE HEMANGIOBLAST STATE

Soo Kim[1], Peter Toth[1], Yanmin Zhang[1], Damiano Rondelli[2], Jalees Rehman[3], Asrar B. Malik[1]
[A]Department of Pharmacology, [B]Section of Hematology/Oncology, [C]Department of Medicine and Pharmacology, [1]University of Ilinois at Chicago, Chicago, IL, United States

Induced pluripotent stem cells (iPSCs) can be generated from mouse and human adult fibroblasts by ectopic expression of the four transcription factors OCT4, KLF4, SOX2 and c-Myc (OKSM). Recent studies have demonstrated that lineage conversion of fibroblasts towards selected lineages cells such as cardiomyocytes or neural progenitors can be achieved by transient expression of OKSM, combined with lineage-directed differentiation factors and thus circumventing a state of complete pluripotency. Here we examined the possibility of transduction of fibroblasts with OKSM followed by exposure to endothelial or hematopoietic growth factors to induce lineage conversion of human fibroblasts into endothelial and hematopoietic cells.
We developed a dedifferentiation and lineage conversion protocol modeled on embryonic development during which both endothelial and hematopoietic cells are derived from multipotent mesodermal hemangioblasts. After one week of OKSM over-expression, 25.2% of partially reprogrammed fibroblasts expressed the hemangioblast marker CD34. Upon analysis of gene expression of reprogrammed fibroblasts at day 7, we found that the expression levels of hemangioblast marker CD34 (7-fold), endothelial transcription factor ER71 (37-fold) and the endothelial surface marker VEGF-receptor-2 (11-fold) were increased compared to fibroblasts. Fibroblast derived $CD34^+$ cells were sorted by FACS and clearly showed the potential to differentiate into either endothelial or hematopoietic lineages. After endothelial cell differentiation, expression levels of the VEGF-receptor -2 were increased even further, and we also observed expression of markers of mature endothelial cells such as CD31 (PECAM), vWF (von Willebrand Factor) and CD144 (VE-cadherin). These highly proliferative fibroblast derived endothelial progenitor cells (Fib-EPCs) demonstrated in vitro angiogenic potential and responded to inflammatory stimuli with upregulation of

endothelial adhesion molecules, thus showing functional responses typically found in endothelial cells. After exposure of fibroblast derived $CD34^+$ cells to hematopoietic growth medium, the expression levels of the hematopoietic transcription factors GATA2, RUNX1, SCL and Pu.1 were increased when compared to control fibroblasts. Also, re-plating of $CD34^+$ cells on methylcellulose generated hematopoietic colonies.
These data indicate that ectopic expression of pluripotency factors can de-differentiate fibroblasts into hemangioblastic $CD34^+$ cells, which in turn can differentiate into endothelial or hematopoietic lineages. Lineage conversion of fibroblasts into both, hematopoietic and endothelial cells has significant therapeutic potential in chronic ischemia. This approach not only represents a useful source for regenerating damaged tissue and engineering vascular tissue engineering, but may also allow for the construction of artificial bone marrow niche-like environments (ECs, HSCs and MSCs) *ex vivo*.

T-2221

DEFINED 3D IN VITRO CULTURE CONDITIONS MODULATE PLURIPOTENCY FACTOR EXPRESSION AND CHROMATIN ORGANISATION IN HUMAN MESENCHYMAL STEM CELLS

Rebecca R. Mason[1], Elen Bray[2], Drew Burdon[3], Paul G. Genever[1]
[1]Biology, University of York, York, United Kingdom, [2]CM Technologies, York, United Kingdom, [3]Smith and Nephew, York, United Kingdom

Mesenchymal stem cells/multipotent stromal cells (MSCs) can be isolated from the bone marrow of patient tissue removed during joint replacement surgery and are traditionally cultured as plastic-adherent monolayers in vitro. However, this simplistic 2D culture method may not accurately model the more complex 3D environment in which MSCs reside in vivo. We sought to characterise the effects of a defined 3D in vitro model on MSC behaviour. MSCs were induced to form spheroid structures by culturing in non-adherent, U-bottomed 96-well plates in the presence of 0.25% methyl cellulose. The nuclear morphology of MSCs cultured as 3D spheroids was dramatically altered when observed by TEM; monolayer MSCs had a regular rounded nuclear morphology, whilst 3D MSC nuclei were irregular and multi-lobed. Given the distorted nuclear morphology of 3D MSCs, we then examined chromatin organisation using immunocytochemistry. Staining for trimethylated lysine 4 of histone 3 (H3K4me3), a euchromatin marker, was enriched throughout 3D MSC nuclei, whilst in 2D MSCs, intense staining was confined to more distinct nuclear regions. Trimethylated lysine 9 of histone 3 (H3K9me3), a marker of heterochromatin, showed diffuse nuclear staining in 3D MSCS, compared to distinct foci which marked heterochromatic regions in 2D MSCs. These data suggested that 3D MSC nuclear architecture and chromatin organisation had altered to an open, transcriptionally permissive state. We screened a panel of different 3D MSC conformations, generated by different initiating MSC seeding number and time in culture, for evidence of pluripotency. When 60,000 MSCs were cultured as a spheroid for 5 days, qRT-PCR revealed up to 46-fold increase of expression of the pluripotency markers Oct4, Sox2 and Nanog, in 3D compared to 2D cultures. However subcutaneous implantation of 3D MSCs into nude mice did not generate teratomas, as observed in embryonic stem cell (ESC) controls. In assays of 3D MSC reprogramming efficiency, ESC-like colonies were observed when MSCs 'primed' by 5 day-3D culture were transduced with lentiviral vectors expressing combinations of Oct4, Sox2, Klf4 and c-Myc. MSCs from donor A (osteoarthritic female, 79 years) produced Oct4a+ colonies at an efficiency of 0.11%, whilst MSCs from donor B (osteoarthritic female, 57 years) produced Oct4a+ colonies at an efficiency of 0.61% when transduced with all four factors. When transduced with just Oct4 and Sox2, MSCs from donor B produced Oct4a+ colonies at an efficiency of 0.22%. Donor B was able to produce a number of ESC-like Oct4a+ colonies when transduced with Oct4 alone, at an efficiency of 0.14%. We propose that our defined 3D in vitro culture model causes MSCs to revert to a more primitive state, with enhanced expression of factors required for pluripotency. These 'primed' 3D MSCs provide a cell source which is easily induced to pluripotency, a process possibly aided by the modulation of nuclear and chromatin organisation, and which could be used as a patient-specific disease model in vitro.

T-2222

DIRECT REPROGRAMING OF LINEAGE COMMITTED CELLS TOWARDS FUNCTIONAL MESENCHYMAL STEM CELLS USING DEFINED FACTORS

Vashe Chandrakanthan[1], Jair Kwan[1], Ashwin Unnikrishnan[1], Dominik Beck[1], Lies Boelen[1], Michael J. Carnell[2], Louise E. Purton[5], Trevor Lewis[3], Jason Wong[1], Luke Hesson[1], Rema A. Oliver[4], William R. Walsh[4], John E. Pimanda[1]
[A]Adult Cancer Program, Lowy Cancer Research Centre, [B]Biomedical Image Facility, Mark Wainwright Analytical Centre, Lowy Cancer Research Centre, [C]School of Medical Sciences, [D]Surgical & Orthopaedic Research Laboratories, Prince of Wales Clinical School, [1]University of New South Wales, Kensington, Australia, [2]St. Vincent's Institute, The University of Melbourne, Kensington, Australia

Exogenous mesenchymal stem cell (MSC) based therapies have been proposed as an alternative or supplement to autografts, allografts or synthetic graft materials to promote bone healing. Reconstituting bone and cartilage by inducing endogenous cell proliferation as an alternative to exogenous cell therapy would significantly benefit the prophylaxis and treatment of osteoporotic fractures, degenerative joint disease as well as traditional fracture healing, non-unions and fusions. Whereas somatic cells have been reprogrammed into embryonic stem cells (ESCs) and tissue specific progenitors by introducing defined transcription factors, whether terminally differentiated cells can be reprogrammed into mesenchymal stem cells (MSCs) is not known. We have identified two factors; 5-azacytidine and platelet derived growth factor-AB (Pdgf-AB), which when used in combination, can reprogram terminally differentiated adipocytes, chondrocytes and osteocytes into functional MSCs. Osteoblasts and osteocytes harvested from compound transgenic mice showed progressive loss of osteogenic markers and acquisition of MSC markers when live imaged in culture in the presence of these factors. These reprogramed cells showed in vitro and in vivo CFU-F activity, including serial clonogenicity and long-term self-renewal, which compare favorably with freshly isolated MSCs. Reprogrammed CFU-Fs also exhibited equivalent multipotency for a range of mesodermal (beating cardiomyocytes, endothelial cells, smooth muscle cells, adipocytes, chondrocytes and osteocytes), endodermal (hepatocytes) and neuroectodermal (depolarizing neurons, astrocytes and oligodendrocytes) cell types. Reprogrammed CFU-Fs did not form teratomas when transplanted under the kidney capsule, and therefore cannot be considered pluripotent by this criterion. Consistent with this finding, early and late passages of reprogrammed CFU-Fs whilst expressing low-levels of some pluripotent markers (Oct4, Sox2, cMyc and Klf4) albeit at levels significantly lower than ESC and comparable with freshly isolated MSCs, lacked expression of others (Rex1 and Nanog). However, when co-transplanted with ESCs under the kidney capsule, reprogrammed CFU-Fs contributed to a broad range of mesodermal, endodermal and ectodermal cell types in support of their broad in vivo plasticity. To support these functional data, we have analyzed genome-wide expression profiles of adipocytes, osteocytes, chondrocytes and their respective reprogrammed CFU-Fs to evaluate the degree of reprogramming. Both hierarchical clustering analysis and pairwise scatterplots showed that the reprogrammed CFU-Fs closely resembled control MSCs, but clearly differ from their parental cell types and ESCs. Our data also showed that following reprogramming, the distinct epigenetic profiles of terminally differentiated cells converge and overlap with that of MSCs. To evaluate the in vivo tissue repair capability of these reprogrammed CFU-Fs, we used a single level lumbar spinal fusion model. Reprogrammed bone fragments fused with host lumbar vertebrae with new bone formation, in contrast with non-reprogrammed fragments, which failed to integrate. Taken together we conclude that terminally differentiated cells can be reprogrammed into MSCs using a defined set of factors and is an effective way of reprogramming cells without using viral vectors and as such has immediate clinical relevance.

T-2223

RELIABLE AND PRACTICAL PROTOCOL TO GENERATE MMRNA-INDUCED PLURIPOTENT STEM CELLS (RIPS)

Eduard Yakubov, Sheena Abraham, John P. Cooke
Stanford, Stanford, CA, United States

Safe DNA-free reprogramming protocols are optimal to
generate iPSCs for therapeutic use. Recently it was shown that mRNA, which is
inherently non-integrative, can be used as a potential tool to generate

clinically useful iPSCs. We have developed a robust and reproducible “working protocol” for generating RNA induced iPS (RiPS). This protocol is based on mmRNA constructs encoding for OCT4, SOX2, KLF4, cMYC, LIN28 which were synthesized by in vitro transcription (IVT) using as a template a set of customized plasmids (pJRG-Oct4, pJRG-Sox2, pJRG-Klf4, pJRG-cMyc, pJRG-Lin28) for in vitro transcription (IVT). Each plasmid encodes 5’ and 3’ UTRs of human beta globin (hBB), which enhances the stability of the mmRNA; a long (150 nt) poly-A tail to further increase stability; and a restriction site at the end of the poly-A tail to allow for linearization before IVT. The restriction site is that of SapI, a type IIs restriction enzyme, which permits the mRNA to terminate in the poly-A tail without any unnecessary non-adenine nucleotides that might compromise mRNA stability. Daily transfection for 10 days with the mmRNA constructs encoding the OKSML factors in adult human fibroblasts maintained on feeder cells generates TRA-1-60+ cells by live staining. These cells are FACS sorted and replated onto Matrigel resulting in efficient colony formation of transgene-free human RiPS.
In addition to these mmRNAs constructs which may be directly synthesized by IVT using as templates our verified set of plasmids, this protocol requires only commercially available molecular biology reagents and cell culture expertise to yield human RiPS colonies from patient specific fibroblasts in approximately 2 weeks.
This technology generates large numbers of patient-specific transgene-free human RiPS cells for regenerative medicine, in vitro studies of disease mechanisms, as well as for drug screening.

T-2224

ENGINEERED TRANSCRIPTION FACTORS FOR CONVERTING HUMAN FIBROBLASTS INTO SPINAL MOTOR NEURONS

Joseph R. Klim, Nick D. Atwater, Justin K. Ichida, Kevin C. Eggan
Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States

Neuronal diversity is key to the mammalian central nervous system’s sophistication. From sensory neurons that collect information to the billions of neurons in the brain that process information to motor neurons (MNs) that execute responses, diverse classes of neurons work together to form a complex neural network. This cellular diversity is achieved during development thorough the combinatorial expression of a surprising limited repertoire of transcription factors. Based on this knowledge, forced expression of neural-lineage-specific transcription factors has been used to convert fibroblasts into neurons. More complex cocktails of transcription factors have also be used to convert fibroblasts into more specialized neural subtypes. For example, our group has demonstrated that a combination of induced neuronal factors (Brn2, Ascl1, Myt1l, and NeuroD1) together with Ngn2, and the LIM-homeodomain family of transcription factors Isl1 and Lhx3 can reprogram fibroblasts into functional spinal motor neurons (iMNs). Isl1 and Lhx3, however, are also expressed by interneurons and sensory neurons. To eliminate the possibility that we are reprogramming fibroblasts into these other neuronal cell types, I employed an Isl1-Lhx3 fusion protein. This Isl1-Lhx3 fusion protein predisposes the transcription factors to form the MN-promoting hexameric complex. To this end, I employed the Isl1-Lhx3 fusion protein in combination with other TFs (Brn2, Ascl1, Myt1l, Ngn2, and NeuroD1) to reprogram fibroblasts into MNs. Our results demonstrate that the MN-tailored transcription factor can replace the individual TFs (Isl1 and Lhx3) to generate iMNs. These iMNs are similar to human embryonic stem cell-derived MNs, but compared to induced MNs generated with 7 factors, a greater percentage of the resulting cells have morphological characteristics of MNs and stain positive for mature neuronal markers (e.g. MAP2). Our data indicate that engineered transcriptional complexes can be used to efficiently transform fibroblasts

into MNs. We anticipate these findings will yield advances in generating human MNs for disease modeling and insight into the molecular mechanisms controlling cellular identity.

T-2225

DERIVATION OF IPSCS WITH HOMEMADE MRNAS: ONE STEP TOWARDS A BETTER GENOMIC INTEGRITY?

Clara Steichen[1], Eleanor Luce[1], Jérôme Maluenda[2], Sylvie Goulinet-Mainot[1], Gérard Tachdjian[3], Anne Weber[1], Deborah Burks[4], Joëlle Marie[5], Judith Meli[6], Anne Dubart-Kupperschmitt[1]
[1]Inserm U972, Villejuif, France, [2]INSERM UMR_S788, Le Kremlin-Bicêtre, France, [3]Department of Cytogenetics, Clamart, France, [4]eCIBER de Diabetes y Obesidad, Centro de Investigación Principe Felipe, Valencia, Spain, [5]Molecular Genetics Centre, CNRS, UPR 3404, Gif-sur-Yvette, France, [6]INSERM UMR_S788, IFR 93, Le Kremlin-Bicêtre, France

Despite the great potential of induced Pluripotent Stem Cells (iPSCs) in cell therapy and disease modeling applications, the genomic integrity of these cells still raises some concerns. Using high throughput genomic analyses, recent advances have been made reporting the occurrence of Copy Number Variations (CNV) or Single Nucleotide Variations (SNV) in iPSCs. Several mechanisms have been reported as being potential drivers of this genomic instability (pre-existing mutations in the initial population, intrinsic instability of pluripotent genomes, cell expansion or direct impact of the reprogramming process). In this view, a common effort is done to define a robust reprogramming protocol minimizing its mutational impact on the iPSC genome. Among non integrative strategies, the use of synthetic messenger RNAs (mRNA) is appealing but little is known about the genomic stability of the derived cells.
Here, we report the successful generation of human iPSCs based on repetitive transfections of homemade mRNAs and their genomic analysis using pangenomic SNP/CNV microarrays. To ensure the robustness of the reprogramming strategy and the reliability of the genomic analysis, we performed two independent experiments with mRNAs encoding OCT4, KLF4, SOX2, cMYC +/- LIN28. Our genomic study was achieved in strict comparison with retroviral-mediated iPSC clones (P1, P2) derived from the same fibroblasts. All cell lines displayed the common features of iPSCs, including teratoma formation capacity. The SNV analysis showed a total number of single point mutations (de novo or loss of heterozygosity) in P1 and P2 four-fold higher than that of RNA-derived clones (mean of 1576 vs. 384). A total number of 67 SNVs appeared to be in exonic regions (between 5 and 29 for each clone). Interestingly, an average number of 9 mutations in coding sequences was observed in virus-derived iPSCs versus only 5 for RNA-derived iPSCs. Results of unsupervised hierarchical clustering of the 67 exonic SNVs showed that RNA-derived iPSC lines clustered in two distinct groups, representing the two mRNA-reprogramming experiments. Moreover, these two groups clustered apart from retroviral-mediated iPSC lines.
The CNV analysis showed the occurrence of 0 to 7 deletions (≥100kb) in all iPSC lines. These deletions did not affect tumor-related genes. Our present study could not reveal any correlation between the reprogramming method and the number, size or localization of the CNVs. These results tend to show that the observed deletions are not directly related to the reprogramming method but are more likely due to a positive selection of cells carrying deletions acquired during reprogramming or present in the initial fibroblast population.
Finally, we tested the hepatic differentiation capacity of mRNA-derived iPSCs. Using previously published protocols, we showed that these cells are able to differentiate into hepatocyte-like cells. These differentiated cells will be studied for their genomic integrity.
Our pangenomic analysis of iPSCs, derived and amplified in strictly comparable conditions with two different reprogramming methods, experimentally shows that the attractive integration-free mRNA reprogramming strategy leads to a better maintenance of the genome integrity.

T-2226

SINGLE-CELL ANALYSIS OF MYOGENIC DIFFERENTIATION REVEALS A BISTABLE LINEAGE COMMITMENT SYSTEM

Tyler M. Gibson, Charles A. Gersbach
Biomedical Engineering, Duke University, Durham, NC, United States

The ultimate success of stem cell therapies for regenerative medicine is dependent on the ability to precisely control cellular lineage commitment. However, cells display a significant degree of heterogeneity in their responses to stimuli that direct differentiation or reprogramming to a target lineage. The changes that occur during cellular lineage commitment are governed by complex gene networks that modulate individual cell responses to differentiation signals and give rise to varied responses in a population of cells. Part of this variable response is intrinsic to the intracellular environment, as genetically identical cells display heterogeneous responses to a uniform differentiation stimulus. A greater understanding of how these stochastic cell responses to differentiation and reprogramming can improve our ability to develop effective gene and cell therapies. Our objective is to study induced lineage commitment at the single-cell level to gain a better understanding of the processes that occur during differentiation. We are using MyoD-driven skeletal muscle lineage commitment as a model pathway of differentiation.
We have measured the expression levels of myogenin, a marker of commitment to terminal myoblast differentiation, using single-cell fluorescence reporter assays and single-molecule mRNA detection. These measurements enabled us to make novel observations regarding the population distribution of primary myoblasts and reprogrammed fibroblasts during induced muscle differentiation. We found that MyoD-driven upregulation of myogenin is a stochastic and bimodal process in differentiating skeletal myoblasts. However, in myoblasts with high levels of myogenin expression, there was a graded and unimodal relationship between MyoD and myogenin expression levels. When exogenous MyoD was expressed at high levels in fibroblasts, we observed a similar unimodal and graded trend, where a higher dose of MyoD led to higher expression of myogenin mRNA. These findings are consistent with the hypothesis of two main attractor states of myogenin expression levels. They also show the cell's transition from a low-myogenin to a high-myogenin state during induced myogenic differentiation and myogenic reprogramming.
These results offer a quantitative single-cell analysis of myogenesis. The quantitative nature of these results will enable in-depth mechanistic modeling of cell-fate commitment during myogenesis. We expect these discoveries will lead to more effective cell-based therapies by offering a deeper understanding of stem cell biology.

T-2227

NAIVE HUMAN PLURIPOTENT STEM CELLS FROM ESTABLISHED HESCS/HIPSCS AND REPROGRAMMING OF SOMATIC CELLS

Alejandro De Los Angeles[1], Peter G. Kim[1], Rui Zhao[1], Samuel Entwisle[2], Shiwei Liu[1], Samantha Morris[1], Stephanie Chou[1], Yuin-Han Loh[3], Laurence Daheron[2], Paul Tesar[4], Alexander Meissner[2], George Q. Daley[1]
*[1]Stem Cell Transplantation Program, Division of Pediatric Hematology Oncology, Children's Hospital Boston, and Dana-Farber Cancer Institute; Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, United States, [2]Harvard University, Cambridge, MA, United States, [3]Epigenetics and Cell Fates Laboratory, A*STAR Institute of Medical Biology, Singapore, Singapore, [4]Case Western Reserve University, Cleveland, OH, United States*

Mouse pluripotent stem cells may exist in two stages: naive or primed, represented by embryonic stem cells (ESCs) or epiblast stem cells (EpiSCs) respectively. Whereas naive pluripotent stem cells self-renew in the presence of LIF-Jak/Stat3 signaling and Mek/Gsk3 inhibitors (2i), the primed state is regulated by bFGF and Activin/Nodal signaling. Human embryonic stem cells self-renew in the presence of bFGF and Activin/Nodal signaling and do not respond to LIF, thereby exhibiting a resemblance to primed pluripotent stem cells. It remains unclear how to stabilize a naive human pluripotent state. To investigate culture conditions that support a naive state, naive mESCs were co-stimulated with LIF/PD0325901/CHIR99021 (2i) and FGF. In the presence of LIF/PD/CH and bFGF, mESCs maintain a naive state, whereas mESCs cultured in bFGF progressed to an EpiSC-like state. LIF/FGF/PD/CH mESCs maintained Oct4 and SSEA1 immunoreactivity and high telomerase activity while maintained features of a naive state. Co-stimulated mESCs maintained a mESC-like doubling time, domed morphology, and marker profile, including upregulation of transcripts for naive markers Klf4, Klf2, Rex1, and Nr5a2 and downregulation of primed marker Fgf5. These data suggest that in the mouse, FGF signaling through Mek-independent pathways does not antagonize the naive state, whereas signaling downstream of Mek specifies the primed state.

To test these principles in established human pluripotent cell lines, we cultured human ESCs in LIF/FGF/PD/CH and SB431542, an inhibitor of ACTIVIN/NODAL signaling, and observed differentiation. Kenpaullone (KP) can replace MEK inhibition in maintaining naive mouse ESCs, but without inhibiting ERK. To move established human ESCs backwards without transgenes, we cultured hESCs in FGF/KP/CH/SB431542,
which enabled maintenance for at least 10 passages. These chemically-reverted hESCs and hiPSCs maintained mESC-like morphology, OCT4, SOX2, and NANOG expression, high telomerase activity, formed teratomas and were amenable to transgenesis because they could be passaged as single cells. Similar MEK-independent cell lines could be obtained in FGF/PD/CH/SB431542, but not LIF/PD/CH/SB431542,
following introduction of KLF4/RARG/NR5A2 into hESCs. These data favor a unique contribution to self-renewal by FGFR signaling in this context.
Derivation of new hESCs/hiPSCs in similar media containing these inhibitors without bFGF will decipher whether FGFR dependence is due to “addictive behavior” owed to conventional hESC/hiPSC derivation conditions or whether FGFR signaling has a fundamental role in human pluripotent stem cell biology. We obtained LIF-dependent naïve human iPS cells (nhiPSCs) independent of FGFR signaling during direct reprogramming of somatic cells, indicating that cell type of origin may influence obligatory dependence on FGFR signaling. The requirement of exogenous transgenes to maintain these nhiPSCs is currently being evaluated. We have thus defined conditions that employ a complex mix of cytokines and signaling pathway inhibitors, with or without additional transcriptional regulators, which maintain a human naïve state of pluripotency. Further enhancement of the core naïve human transcriptional circuitry will facilitate efficient generation of high-quality nhiPSCs.

T-2228

REPROGRAMMING ACTIVITY OF NANOGP8, A NANOG FAMILY MEMBER WIDELY EXPRESSED IN CANCER

Adelaida R. Palla, Daniela Piazzolla, Maria Abad, Han Li, Orlando Dominguez, Helia B. Schönthler, Erwin F. Wagner, Manuel Serrano
Molecular Oncology, Spanish National Cancer Research Center CNIO, Madrid, Spain

NANOG is a key transcription factor for pluripotency in embryonic stem cells. The analysis of NANOG in human cells is confounded by the presence of multiple and highly similar paralogs. In particular, there are three paralogs encoding functional proteins, namely, *NANOG1*, *NANOG2* and *NANOGP8*, and at least eight additional paralogs that do not encode full-length NANOG proteins. Here, we have used a multi-*NANOG* PCR that amplifies the three functional paralogs together with most non-functional ones to examine *NANOG* expression in human embryonic stem cells (hESCs) and in human cancer cell lines. As anticipated, we found that hESCs express large amounts of *NANOG1* and, interestingly, they also express *NANOG2*. In contrast, most human cancer cells tested express *NANOGP8* and the non-coding paralogs *NANOGP4* and *NANOGP5*. Notably, in some cancer cell lines, the NANOG protein levels produced by *NANOGP8* reach up to 10% of the NANOG protein levels in pluripotent cells. Given the widespread expression of *NANOGP8* in cancer cells and the fact that its encoded protein only differs in 2 amino acid residues from *NANOG1*, we tested whether *NANOGP8* is functional. We show that *NANOGP8* is as active as *NANOG1* in the reprogramming of human and murine fibroblasts into induced pluripotent stem cells (iPSCs). These results demonstrate that most cancer cells possess NANOG activity that could contribute to promote de-differentiation and/or cellular plasticity.

T-2231

SCREENING EMBRYONIC STEM CELL FACTORS IDENTIFIES A NOVEL CLASS OF PROTEINS INVOLVED IN REPROGRAMMING

Stan Wang, Kei Miyamoto, John B. Gurdon
Wellcome Trust/CRUK Gurdon Institute, University of Cambridge, Cambridge, United Kingdom

The ability to reprogram differentiated cells holds great promise for use in regenerative medicine. However, direct application in human therapy is limited by issues of efficiency, aberrant genetics, and a changing understanding of

the epigenetic landscape. In order to improve reprogramming, we screened an mRNA library derived from mouse embryonic stem cells using injected Xenopus oocytes to find factors that upregulate the expression of pluripotency genes in co-injected mouse embryonic fibroblasts. Through this, we identified a single factor, ribosomal protein S26 (Rps26) that was seen to upregulate key pluripotency markers in a time- and dose-dependent manner. Of note, Rps26 is highly conserved across species and shows differential expression during early mouse embryonic development via GEO Profile analysis. Rps26 has been demonstrated to interact with its own pre-mRNA and is deficient in Diamond-Blackfan Anemia. Furthermore, recent studies have shown that ribosomal proteins can have not only transcript-dependent translational control, but are increasingly being found to have extraribosomal functions in gene regulation. Overall, these results suggest a potential role for ribosomal proteins, as a novel class of proteins, in nuclear reprogramming. As such, we aim to test the effect of Rps26 in iPS cell reprogramming. If successful, this would demonstrate the utility of screening for factors via nuclear transfer with direct consequences in other reprogramming systems.

T-2232

REPROGRAMMING DIABETIC FOOT ULCER-DERIVED FIBROBLASTS TO IMPROVE CHRONIC WOUND REPAIR

A. G. Maione[1], B. Gerami-Naini[2], L. Park[2], A. Smith[1], Vanessa Yanez[2], F. Tecilazich[3], E. Leal[3], A. Gianotti-Smmer[4], G. Mostoslavsky[4], A. Veves[3], C. Kearney[5], D. J. Mooney[5], **J. A. Garlick**[6]

[1]Tufts University School of Medicine, Sackler School of Graduate Biomedical Sciences, Boston, MA, United States, [2]Dept. of Oral and Maxillofacial Pathology, Tufts University, Boston, MA, United States, [3]Harvard Medical School, Beth Israel Deaconess Medical Centeer, Boston, MA, United States, [4]Boston University School of Medicine, Center of Regenerative Medicine (CReM), Boston, MA, United States, [5]Division of Engineeering and Applied Science, Harvard University, Cambridge, MA, United States, [6]School of Dental Medicine, Dept. of Oral and Maxillofacial Pathology, Boston, MA, United States

Diabetic foot ulcers (DFU) are a common complication of diabetes that can lead to limb amputation. Unfortunately, current treatments fail to promote healing in roughly 50% of patients. Fibroblasts are an essential cell type in wound healing due to their production of extracellular matrix (ECM), secretion of cytokines and support of re-epithelialization. Yet, it is poorly understood how altered fibroblast function contributes to impaired healing of DFUs and if these alterations can be reversed. Studies in other cell types have demonstrated that epigenetic patterns are altered in diabetic cells. Thus, we hypothesized that the epigenetic signatures of DFU fibroblasts may be linked to their impaired wound healing potential. Previous work in our lab has demonstrated that the directed differentiation of iPSC to a fibroblast fate improves their wound repair potential (Shamis *et al.,2012).* Our objective in the current study is to determine if epigenetic changes occurring during reprogramming can direct DFU fibroblasts from a "repair deficient" to a "repair competent" phenotype.

In the current study, we first compared the wound healing profiles of 12 primary fibroblast cell lines derived from DFUs to non-diabetic fibroblasts (NFF) and diabetic fibroblasts (DFF) taken from the dorsum of the foot. We characterized these lines using 2D culture assays (ELISA, RT-PCR, growth curves) and 3D engineered-tissue assays (production of ECM and support of skin re-epithelialization) to guide our choice of fibroblasts for reprogramming to iPSCs using the STEMCCA polycistronic lentiviral vectors containing Oct4, Sox2, Klf4, cMyc (Somers *et al.,*2010). We performed microarray analysis (Illumina BeadChip) and whole genome bisulfite sequencing on these cells to compare gene expression and DNA methylation patterns before reprogramming, after reprogramming to iPSCs and following differentiation to a fibroblast fate.

We found that DFU and DFF cells underwent replicative senescence before NFFs and produced less ECM than NFFs in our 3D, *in vitro* assay. DFU and DFF cells produced less IL-6 when compared to their normal counterparts. Following *in vivo* transplantation of DFU, DFF and NFF cells into the wounds of non-diabetic mice, we observed NFF cells accelerated wound closure compared to DFU cells. Reprogramming efficiencies of diabetic cell lines varied from patient to patient, with efficiencies ranging from 0.01%-1%. Microarray analysis revealed that DFU and DFF samples were clustered and were distinct from the NFF. Whole genome bisulfite sequencing analyses using the Illumina HiSeq2000 are currently underway to investigate the DNA methylome at base pair resolution. We are presently

studying whether epigenetic alterations in iPSCs derived from DFUs can acquire repair-promoting functions when they are subsequently differentiated to a fibroblast fate. By revealing which functions and genes are altered in DFU fibroblasts upon reprogramming, we hope to gain insight into the molecular mechanisms that drive chronic wound formation to develop novel and more effective therapies for chronic diabetic wounds.

T-2233

MOLECULAR CHARACTERIZATION OF MYT1L DURING DIRECT REPROGRAMMING OF MOUSE EMBRYONIC FIBROBLAST TO INDUCED NEURONAL CELLS

Moritz Mall, Daniel Fuentes, Thomas Vierbuchen, Soham Chanda, Marius Wernig
Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA, United States

Forced expression of transcription factors is able to directly reprogram distant cell types into another. Recently, our lab showed that expression of the neuronal transcription factors Ascl1, Brn2 and Myt1l can convert mouse embryonic fibroblast to induced neuronal (iN) cells. While the functions of Ascl1 and Brn2 during neural development have been extensively investigated, relatively little is known about Myt1l (myelin transcription factor 1-like; also known as Nzf1 or Png1).
Myt1l is a zinc finger transcription factor with six C2HC-type zinc finger domains that has been identified as one of the earliest specific markers of postmitotic neurons. Interestingly, Myt1l is one of the few characterized neuronal transcription factors that is expressed not only in the central but also in the peripheral nervous systems, indicating that it might regulate aspects of pan-neuronal identity
Here we identify the molecular domains of Myt1l that confer reprogramming capacity by showing that a deletion mutant containing only these minimal domains fully recapitulates the efficiency and maturity of neuronal reprogramming that is seen using wild type Myt1l. We show that neuron-like cells generated with Ascl1 and this Myt1l deletion mutant do not differ functionally or morphologically from those generated with Ascl1 and wild type Myt1l. Our data therefore provides molecular insight into functional domains of Myt1l that mediate reprogramming of fibroblast into functional neurons.

T-2234

GENERATION OF TRANSGENE-FREE IPSC LINES FROM PARKINSON'S DISEASE PATIENTS

Kun Bi[1], Spencer B. Hermanson[1], Connie Lebakken[1], Marian Piekarczyk[1], Tori Barron[1], Laurie Reichling[1], Kurt Vogel[1], J. William Langston[2], Birgitt Schuele[2]
[1]Primary and Stem Cell Systems, Life Technologies, Madison, WI, United States, [2]The Parkinson's Institute, Sunnyvale, CA, United States

Patient-derived induced pluripotent stem cells (iPSCs) offer exciting potential in both cell therapy and *in vitro* disease modeling. Efficient reprogramming of patient somatic cells to iPSCs plays a key role in realization of this potential. Many reprogramming methods have been optimized for use with cell lines or robust primary cells, but lead to technical challenges in converting adult or disease somatic cells to iPSCs consistently and with high efficiency. Traditional methods relying on integrating virus or plasmid to reprogram could potentially result in multiple insertions and risk of tumorigenicity. Reprogramming with episomal vectors, mRNAs and miRNAs typically leads to low reprogramming efficiency or requires multiple rounds of transfection.
Sendai virus is a negative-strand RNA virus that replicates in the cytoplasm of infected cells and does not integrate into the host genome. Recent literature demonstrates that Sendai virus delivering the four Yamanaka factors is a highly efficient method to reprogram normal human foreskin fibroblasts, peripheral blood mononuclear cells and CD34+ cells to generate integration-free iPSCs.
In these studies, fibroblasts from skin biopsies of four Parkinson's disease (PD) patients and two age-matched control individuals were efficiently reprogrammed to iPSCs using the Sendai reprogramming method. These iPSCs are transgene-free and karyotypically normal, express known pluripotency markers and are able to differentiate into embryoid bodies that present three germ layer lineages. Gene expression profiles clearly distinguished these iPSCs

from their parental fibroblasts and demonstrate a high level of consistency with expression patterns from control iPSCs and H9 ESC line. Given the efficiency, speed and ease with which we were able to reprogram adult disease fibroblasts, we anticipate the Sendai reprogramming method being applied to large scale reprogramming of multiple disease lines potentially in an automated fashion.

T-2235

COMPARISON OF METHYLATION STATUS AND GENE EXPRESSION IN ISOGENIC HUMAN EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS

Barbara S. Mallon[1], Rebecca S. Hamilton[1], Olga A. Kozhich[2], Kory R. Johnson[2], Mahendra S. Rao[3], Yang C. Fann[2], Pamela G. Robey[4]

[A]NIH Stem Cell Unit, [1]NINDS/NIH/DHHS, Bethesda, MD, United States, [2]NIAMS/NIH/DHHS, Bethesda, MD, United States, [3]NIDCR/NIH/DHHS, Bethesda, MD, United States

Since the advent of induced pluripotent stem cell (iPSC) technology, there has been tremendous interest in reprogramming somatic cells. However, the extent to which such iPSCs faithfully recapitulate the characteristics of embryonic stem cells (ESCs) remains a subject of debate. Previously, a Human Pluripotent Stem Cell Database at the National Institutes of Health was established that provides a comprehensive, quality-controlled reference dataset of pluripotent human cells (http://stemcelldb.nih.gov/). Cross-comparison of the pluripotent cells contained in that dataset has previously demonstrated that there exists no exclusive gene expression profile between hESCs and hiPSCs. In fact, many differences reported elsewhere are most likely due to natural human genetic variation. In the current study, we have derived a differentiated population of dividing neural precursor cells (NPCs) from the H1 (WA01) human embryonic stem cell line using an efficient embryoid body mediated protocol primarily based on the use of noggin and bFGF. The NPCs generated were negative for the pluripotent stem cell markers SSEA4 and Tra-1-60 and greater than 95% positive for neural cell adhesion molecule (NCAM) as determined by FACS analysis. These cells were subsequently reprogrammed using lentiviral transduction of the four Yamanaka reprogramming factors to generate isogenic hiPSC lines. Six hiPSC lines were analyzed and found to be negative for NCAM and positive for SSEA4 and Tra-1-60 by FACS analysis and positive for Oct3/4 by immunostaining. Three such lines have been extensively characterized by gene expression and methylation profiles in addition to standard pluripotency tests. All three lines have been confirmed as being isogenic to the parental H1 line by STR analysis and have normal karyotypes. To date, two lines have been tested and shown to have the potential to differentiate into cells representative of the 3 germ lineages by in vitro methods. Analysis of global gene expression and methylation patterns shows variation between the intermediate population and the pluripotent cell populations but a lack of significant differences between the iPSCs and their isogenic hESC counterparts. The elimination of any genome-specific variation in this study confirms the conclusions drawn in our previous work showing no significant differences between hESCs and hiPSCs at the gene expression level.

T-2236

FROM GENES TO THOUGHTS: A CELL REPROGRAMMING-BASED APPROACH TO NEURODEVELOPMENTAL DISORDERS CAUSED BY GENE DOSAGE IMBALANCES

Giuseppe Testa[1], Sina Atashpaz[2], Antonio Adamo[2], Pierre-Luc Germain[2], Josh Chenoweth[3], Giuseppe D'Agostino[2], Matteo Zanella[2], Paolo Prontera[4], Christian Unger[5], Peter W. Andrews[5], Ron D. McKay[3], Giuseppe Merla[6]

[A]Experimental Oncology, [1]European Institute of Oncology, Milan, Italy, [2]Lieber Institute for Brain Development, Baltimore, MD, United States, [3]Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Perugia, Italy, [4]Biomedical Sciences, University of Sheffield, Sheffield, United Kingdom, [5]Medical Genetics Unit, Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Italy

Transcription factor (TF)-induced somatic cell reprogramming is enabling a paradigm shift in our ability to model human diseases, overcoming the fundamental limitation that has been so far the scarce availability of primary diseased tissues, which is particularly salient for disorders of the nervous system. The consequence of this shortage has been the use of transformed lines or cells that are unrelated to the lineages targeted by disease. TF-induced cell reprogramming is overcoming this limitation at an unprecedented pace through the possibility of obtaining from any patient a virtually endless supply of disease-relevant cell-types. Progress promises to be particularly relevant for several neural diseases, for which patient-specific iPSC are being used to elucidate pathogenesis and test new therapies.
Yet, despite the tantalizing possibilities enabled by this approach, there are several fundamental questions that still need to be answered in order to secure iPSC-modeling as a robust platform for the meaningful interrogation of diseases affecting the nervous system. These pertain to two main issues: i) the range of inter-patient and inter-clone variability; and ii) the extent to which already the pluripotent state can be mined to predict gene network alterations in disease-relevant lineages. The former will define our ability to reveal specific derangements against the backdrop of human genetic variation, while the latter would enable rapid progress even before that challenging long-term neuronal differentiation assays are validated on large enough numbers of samples.
Here we address these issues in the case of neurodevelopmental diseases caused by symmetric gene dosage imbalances at 7q11.23: Williams Beuren Syndrome (WBS) and the 7q11.23 microduplication associated to autistic spectrum disorder (7dupASD). The hallmark of WBS is a unique behavioral-cognitive profile that combines hypersociability with a form of intellectual disability characterized by comparatively well-preserved language abilities coupled to severe impairments in visuospatial processing. Hence, the striking symmetry in genotype and phenotype between the two conditions points to this gene cluster as a surprisingly small subset of dosage-sensitive genes affecting social behaviour, language and cognition. As 7q11.23 harbors several transcription factors, we focused our functional dissection of these complementary diseases at the level of transcriptional deregulation in both iPSC and neural progenitors. To this end we assembled a unique cohort of typical WBS, atypical WBS and 7dupASD patients (along with unaffected relatives), and used mRNA reprogramming to establish and characterize at least 3 independent iPSC lines from a total of 12 individuals. Along with the high-resolution assessment of their genomic integrity, this constitutes the largest set of disease samples reprogrammed so far and allowed us to define both the extent of variability and the predictive power of the pluripotent state for disease-relevant traits. We integrated mRNA sequencing with novel tools for reverse-engineering of gene networks and validated results in differentiation assays. This revealed critical transcriptional derangements in disease-relevant pathways that mirror precisely 7q11.23 dosage already in the pluripotent state, and whose validation underscores the importance of sample size and establishes the meaningful interrogation of the pluripotent state for disease modelling.

T-2237

RETROVIRUS INSERTIONS IN IPSC IDENTIFY GENES WHICH FACILITATE SOMATIC REPROGRAMMING

Ali Nowrouzi[1], Takashi Aoi[2], Friederike Herbst[1], Annette Deichmann[1], Anne Arens[1], Phillipp Zimmermann[1], Ulrich Abel[1], Jochen Utikal[3], Hanno Glimm[1], Manfred Schmidt[1], Shinya Yamanaka[2], Christof von Kalle[1]
[1]NCT Heidelberg/DKFZ, Heidelberg, Germany, [2]Center for iPSC Research and Application (CiRA), Kyoto, Japan, [3]German Cancer Center, Heidelberg, Germany

The Identity and molecular function of additional genes that promote reprogramming are relevant for understanding the mechanism facilitating gain-of-pluripotency. Such factors are largely unknown and would be highly relevant for understanding roadblocks to this process. Factor transfer with insertional vectors is one of the most effective reprogramming methods, indicating that vector induced changes might enhance reprogramming. By dissecting the complete insertional inventory of 16 mouse and 24 human iPSC, we show that vector insertions in iPSC were intensely nonrandomly selected in proximity to genes active in embryonic stem cell (ESC) and iPSC expression signatures. We found that insertional activation of mechanistically relevant genes significantly facilitates cellular reprogramming into iPSC. Sequential stages of iPSC generation were enhanced up to 11-fold by forced expression of the identified genes. Genes found to facilitate reprogramming included chromatin modifiers, innate immunity regulat-

ors and RNA Polymerase III regulators. Functional analysis of these genes indicates that stochastic and deterministic roadblocks accompanying reprogramming can be identified and overcome towards more efficient and controlled iPSC derivation.

T-2238

GLOBAL DE-NOVO DNA METHYLATION OCCURS IN ALL CELL SPECIFIC GENES DURING REPROGRAMMING INTO HUMAN PLURIPOTENT CELLS

Jonathan Nissenbaum[1], Ori Bar Nur[3], Eyal Ben David[2], Nissim Benvenisty[1]
[A]Genetics and Stem cell Unit, [B]Genetics, [1]The Hebrew Univ of Jerusalem, Jerusalem, Israel, [2]Cancer Center and Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA, United States

Molecular reprogramming of somatic cells into human induced pluripotent stem cells (iPSCs) is accompanied by extensive changes in gene expression patterns and epigenetic marks. The fashion by which the epigenetic changes execute the silencing of the somatic cell identity and endow a pluripotent stem cell features is not yet fully elucidated. To address this issue and better understand the relationship between gene expression and DNA methylation following molecular reprogramming, we have profiled human somatic and pluripotent cells from three different embryonic layers: 1. Mesoderm - skin fibroblast were reprogrammed to create fib-iPS cells 2. Endoderm - beta cells were extracted from pancreatic islets and had been reprogrammed into beta-iPS (BiPS) cells and 3. Germ cells - a unique resource of parthenogenetic iPS cells were generated from teratomas. The utilization of whole genome expression and DNA methylation profiles enables us to create a comprehensive correlation of the global expression state and the epigenetic state of the cells. In order to refine our resolution on the types of genes that overcome epigenetic changes, we defined four groups of genes based on their expression before and after the reprogramming: genes that are expressed in both somatic and reprogrammed cells, genes that are silenced or activated during reprogramming, and genes that are silenced in both cell types. Each group of genes was analyzed separately for their methylation status upon reprogramming. As for the methylayion analysis, our platform enabled the separation of the epigenetic data to CpG-poor, or CpG-rich island regions. We show that reprogramming is accompanied by extensive increase in DNA methylation mainly in the CpG-poor, non-island promoters. Downregulation of expression of the somatic genes occurred concomitantly with uprgulation in DNA methylation. Intriguingly, DNA methylation of CpG-poor promoters occurred not only for downregulated genes, but also for genes that are not expressed in the parental somatic cells or their respective iPSCs. These genes are predominantly tissue-specific genes of other cell types from different lineages. Our results suggest that DNA hyper-methylation occurs during reprogramming in a global nonspecific fashion on all tissue specific genes from all lineages, regardless of their expression in the parental somatic cells.

Regeneration Mechanisms

T-2241

LOOKING BEHIND THE CURTAIN: PLASTICITY OF POSTNATAL CARDIOMYOCYTES OF THE MOUSE

Alexandra Raulf[1], Caroline Geisen[1], Sabine Grünberg[1], Patricia Freitag[1], Alexandra Klein[1], Wilhelm Roell[2], Bernd Kurt Fleischmann[1], Michael Hesse[1]
[A]Physiology I, [B]Cardiac surgery, [1]University of Bonn, Bonn, Germany

The mammalian heart was generally thought to be a postmitotic organ, but recent findings suggest that there is cardiac turnover in the human heart (Bergmann et al., 2009). However, the source of this cardiomyocyte renewal, which could be either resident cardiomyocyte cell division and/or stem-/ progenitor cell contribution, remains elusive.
The aim of this project is to explore the plasticity of the murine heart by characterizing the cell cycle status and DNA content of cardiomyocytes during postnatal development and after cardiac injury.

To monitor cell cycle progression of cardiomyocytes in vivo and to visualize the onset of physiological cell cycle variations postnatally, we use the eGFP-anillin system (Hesse et al., 2012). This system visualizes cytokinesis and midbody formation as hallmarks of cell division. Live tracking of trophoblast-giant cells, which are known to undergo endoreduplication, revealed the feasibility of the eGFP-anillin system to monitor cell cycle variations as well. The analysis of eGFP-anillin fluorescence in cardiomyocytes post-injury (0-5 eGFP-anillin+ cardiomyocytes per mm2 borderzone at day 4 and day 12 post injury) indicated that few border zone cardiomyocytes of the adult mouse are able to re-enter the cell cycle, but undergo endoreduplication and not cell division. Using high resolution microscopy of postnatal heart cultures, we are currently monitoring the cell biological processes of endoreduplication and acytokinetic mitosis. However, the unequivocal identification of cardiomyocyte nuclei is a major limitation for this type of studies. For this purpose we established an in vivo reporter system for cardiomyocyte nuclei in ES cells and mice using a fusion protein consisting of the human histon 2B and the fluorescence protein mCherry under control of the αMHC promoter (αMHC-H2B-mCherry). A variety of different approaches demonstrates the specificity of expression in cardiomyocyte nuclei and also reveals the technical problems of identifying cardiomyocytes in tissue slices and in vitro using conventional techniques.
By combining the anillin and the H2B transgenic models, we can examine the plasticity of mono- and binuclear cardiomyocytes and hopefully get insight into mechanisms underlying the terminal differentiation of cardiomyocytes.

T-2242

IMPLICATION OF SDF-1 ISOFORMS: SDF-1ALPHA, BETA AND GAMMA IN IRRADIATED DERMAL WOUND HEALING PROCESS

TELMA ROQUE[1], claire SQUIBAN[1], valerie BUARD[1], paméla GASSE[2], Jean Sebastien SILVESTRE[3], marc BENDERITTER[1], Fernando ARENZANA SEISDEDOS[2], **radia TAMARAT**[1]
[1]IRSN, FONTENAY AUX ROSES, France, [2]PASTEUR, PARIS, France, [3]INSERM, HOPITAL HEGP, France

Objective: The wound healing response to experimental injury involves the mobilization and the recruitment of circulating vascular progenitor cells from the bone marrow. In our study, we investigated the SDF-1 alpha, beta and gamma isoforms in wound healing process and in the BMMNC related effects on physiological and pathological wound healing.
Methods: A full thickness excision wound was created by removal of the skin on the midback of irradiated (20Gy) and non-irradiated animals. Using SDF-1 isoforms plasmids, we evaluated angiogenesis and BMMNC mobilization on wound healing process in non-irradiated and irradiated condition.
Results: In non-irradiated condition, we revealed that after skin injury, SDF-1 plasma level was up-regulated by 4-fold compared to non-injured skin. Interestingly, irradiation associated or not with punch affects SDF-1 plasma expression at similar levels.
Total SDF-1 and different isoforms were quantified by RT-PCR in wounded tissue. We demonstrated that in non-irradiated condition, SDF-1 and mainly SDF-1α expression were up-regulated by 50-fold after skin injury compared to control animals. In contrast in irradiated animals, we observed down-regulation of SDF-1α expression in both condition with or without skin injury compared to non-irradiated skin injury. Interestingly, we observed that SDF-1β and SDF-1γ expression were only increased after non-irradiated skin injury compared to control. More interestingly, these results were associated to an up-regulation of CXCR4 receptor after irradiated or not skin injury compared to control animals, whereas irradiation alone had no effect. No modification of CXCR7 expression was observed in each condition.
Finally, using chimeric mice C57BL6 transplanted with BMMNC GFP cells, we evaluated the role of SDF-1 in BMMNC mobilization in irradiated and non-irradiated skin lesion by localization, quantification of GFP cells.
Conclusions: SDF-1alpha participates to dermal wound healing in physiological and pathological condition. This effect is associated to stimulation of angiogenesis and vasculogenesis.

T-2243

FIBROBLAST GROWTH FACTOR 2 INDUCED BY ENRICHED ENVIRONMENT ENHANCES ANGIOGENESIS AND MOTOR FUNCTION IN CHRONIC HYPOXIC-ISCHEMIC BRAIN INJURY

Jung Hwa Seo[1], Ji Hea Yu[2], Ji Yong Lee[2], Yang Hyun Cho[3], Sung-Rae Cho[3]
[A]Graduate Program of Nano Science and Technology, [B]Department and Research Institute of Rehabilitation Medicine, [C]Department & Research Institute of Rehabilitation Medicine, [1]Yonsei University, Seoul, Korea, Republic of

Objective: We investigated the effects of enriched environment (EE) on promoting angiogenesis and neurobehavioral function in an animal model of chronic hypoxic-ischemic (HI) brain injury.
Methods: HI brain damage was induced in seven-day-old CD-1® (ICR) mice by unilateral carotid artery ligation and exposure to hypoxia (8% O2 for 90 min). At six weeks of age, the mice were randomly assigned to either EE or standard cages (SC) (n=15 each). EE mice were housed in a large cage (86×76×31 cm) containing novel objects such as tunnels, shelters, toys, and running wheels for voluntary exercise. The cages also allowed for social interaction (12-15 mice/cage), and the mice were housed in these cages for up to two months. SC controls, however, were housed for the same time duration in a standard cage (27×22.5×14 cm) without social interaction (4-5 mice/cage). Rotarod, forelimb-use asymmetry, and grip strength tests were performed to evaluate neurobehavioral function. In order to identify growth factors that are regulated by EE, an array-based multiplex ELISA assay (Quantibody® array, RayBio-tech, Norcross, GA) was used to measure the expression of the following 10 cytokines and growth factors: fibroblast growth factor-2 (FGF-2), epidermal growth factor, hepatocyte growth factor, insulin like growth factor-1, leptin, matrix metalloproteinase-2, stromal cell-derived factor-1, vascular cell adhesion protein-1, vascular endothelial growth factor, and granulocyte colony-stimulating factor. The expression of FGF-2 in tissue lysate of the frontal cortex and the striatum was also analyzed using western blots. We evaluated platelet endothelial cell adhesion molecule-1 (PECAM-1) and α-smooth muscle actin (α-SMA) using immunohistochemistry.
Results: We found that mice exposed to the EE showed significant improvements in rotarod performance compared to SC controls by post-treatment 8 weeks (132.11±35.29 sec versus 29.50±14.00 sec at 48 rpm; 112.54±34.65 sec versus 15.34±4.59 sec at 56 rpm, p = 0.014 each). In the ladder walking test, the percentage of slips on the transverse rungs of the ladder relative to the total number of steps by the hemiplegic forelimbs was significantly decreased in the EE mice (-4.09 ± 0.95 %) compared to the SC controls (2.07 ± 0.75 %) (p < 0.001). Of the various growth factors examined by multiplex ELISA, the level of FGF-2 was significantly higher in the frontal cortex of EE mice (566.0 ± 262.81 pg/ml) compared with SC controls (211.06 ± 19.47 pg/ml) in the frontal cortex at 8 weeks after treatment (p = 0.043). Likewise, western blot analysis also showed an increase in FGF-2 at 8 weeks after treatment (p = 0.01). On the other hand, in the striatum, FGF-2 expression level increased at 2 weeks after exposure EE (p = 0.01). Immunohistochemistry showed a significantly increased number of α-SMA$^+$ cells at 8 weeks after treatment and expression of PECAM-1$^+$ cells increased and maintained until 8 weeks after treatment in the frontal cortex of EE mice (p = 0.037). The densities of α-SMA$^+$ and PECAM-1$^+$ cells were significantly higher in the striatum of EE treated mice than in control group at both 2 weeks (α-SMA$^+$, p = 0.021; PECAM-1$^+$, p = 0.001) and 8 weeks after treatment (α-SMA$^+$, p = 0.009; PECAM-1$^+$, p = 0.000).
Conclusion: EE enhances endogenous angiogenesis and neurobehavioral functions mediated by the mechanism of astroglial activation and upregulation of FGF-2 in both frontal cortex and striatum of chronic HI brain.

T-2244

EPIGENETIC CHANGES IN COCHLEAR PROGENITOR CELL DIFFERENTIATION TO HAIR CELLS

Dunia Abdul-Aziz, Albert Edge
Otolaryngology, Harvard, Boston, MA, United States

Loss of sensory cells in the ear due to excessive noise, drugs, disease or aging results in deafness. Thus far, deafness is permanent as the adult mammalian organ of Corti lacks the capacity to regenerate hair cells. Herein, we investigate mechanisms underlying cellular differentiation with an eye toward hair cell regeneration. Recently, a pool of hair cell progenitor cells, marked by expression of Leucine-rich repeat-containing G-protein coupled receptor 5

(Lgr5), has been identified among the inner ear supporting cells. These cells have the capacity to self-renew and to differentiate into hair cells. Expression of Atonal 1 (Atoh1), a key developmental gene, is both necessary and specific for specifying a hair cell fate.
Epigenetic regulation, related to the remodeling of chromatin structure via histone methylation and acetylation and DNA methylation, plays an important role in self-renewal, maintenance and differentiation; however, the mechanisms underlying epigenetic control in the inner ear are unknown.
We seek to test a model in which an epigenetically bivalent Atoh1 locus in undifferentiated inner ear progenitor cells acquires activating marks and loses inhibitory marks in the differentiated state. Herein, we present changes in bivalent histone marks--the inhibitory H3K27 methylation and activating H3K4 methylation and H3K9 acetylation--at the Atoh1 locus in undifferentiated and differentiated Ventral organ of Corti (VOT) cells, an immortalized cell line derived from the sensory epithelium of the inner ear which, when placed in differentiating conditions, increases Atoh1 and Myosin VIIa expression--two markers characteristic of hair cells. We examine the potential for epigenetics to influence transcription and cell lineage commitment in the inner ear by investigating the effect of histone modifying agents on the ability to induce hair cell differentiation in a neonatal organ of Corti explant system. This investigation is the first to establish the epigenetic signature at the Atoh1 locus among undifferentiated and differentiated inner ear cells and to investigate the role of epigenetics in hair cell fate determination in the inner ear.

T-2245

SOX2 IS A KDM6A/B TARGET IN HUMAN MESENCHYMAL STEM CELLS: IMPLICATIONS FOR THE REGENERATIVE PROCESS AFTER BIOARTIFICIAL TRACHEOBRONCHIAL TRANSPLANTATIONS

Nina Heldring[1], Vanessa Lundin[2], Philipp Jungebluth[3], Guido Moll[4], Evren Alici[4], Katarina LeBlanc[4], Paolo Macchiarini[3], Ana Teixera[5], Ola Hermanson[1]
[A]Neuroscience, [B]Cell and molecular Biology, [C]Advanced Center for Translational Regenerative Medicine, [D]Medicine and Laboratory Medicine, [E]Cell and Molecular Biology, [1]Karolinska Institutet, Stockholm, Sweden

Sox2 is a transcription factor that exerts reprogramming activities and is a well-known marker for various stem cell populations. We have previously reported that one patient that underwent tracheobronchial transplantation with a bioartificial nanocomposite immersed in the patient's own bone marrow-derived mesenchymal stem cells (MSCs) showed a dramatic increase in Sox2 expression in the peripheral blood mononuclear cells (PBMC) several days after the transplantation. Here we show that increased Sox2 expression coinciding with an increase in CD105+ MSCs was reproducibly observed at around day 6 in the PBMC of a series of three patients receiving different types of bioartificial nanocomposites replacing the tracheobronchial junction. The increased expression correlated in all patients with a decrease in tri-methylated lysine 27 on histone H3 (H3K27me3) of the Sox2 promoter, an enzymatic modification associated with Polycomb-mediated repression of transcription. Notably, we found that these events correlated with an increased expression of the H3K27 demethylases KDM6A/UTX and KDM6B/JMJD3 in the PBMC of the transplanted patients. KDM6B bound directly to the Sox2 promoter and treatment with the specific KDM6A/B inhibitor GSK-J4 resulted in increased H3K27me3 in the Sox2 promoter in patient-derived MSCs. Intriguingly, retinoic acid (RA) treatment of patient-derived MSCs resulted in an increase in KDM6A/B as well as Sox2 expression and this Sox2 increase was inhibited by the demethylase inhibitor GSK-J4. Our results suggest that RA-sensitive KDM6A/B are key regulators of Sox2 expression in PBMC of patients that have undergone transplantations of synthetic tracheobronchial composites, and thus may play an essential role in the regenerative process after the transplantation.

T-2246

NEW BONE FORMATION IN RATS IS ENHANCED BY IMPLANTATION OF HUMAN MESENCHYMAL STEM CELL BUT IS NOT DEPENDENT ON ITS DIFFERENTIATION.

Luciane P. Capelo[1], Bruno V. P. Almada[1], Roberto D. Fanganiello[1], Daniela F. Bueno[3], Gerson S. Kobayashi[1], Marilia T. Martins[2], Maria Rita Passos-Bueno[1]

[A]Dep. of Genetics and Evolutionary Biology, [B]School of Dentistry, [1]University of Sao Paulo, Sao Paulo, Brazil, [2]Hospital Sirio Libanes, Sao Paulo, Brazil

Critical bone defects are observed in many congenital malformations and can also be a result of a disease or accident. The reconstruction of these bones still represents a challenge to surgeons and scientists from the regenerative field. The use of bone marrow stem cells (BMSC) and autologous grafts are currently in use as treatment options for critical bone defects, however, isolation of BMSC or iliac bone confers site morbidity to the donor. In a previous study we characterized the stem cells obtained from orbicularis oris muscle (OOMSCs). These cells can be extracted from patients that are submitted to surgical repair. Here we investigate the osteogenic potential of OOMSCs in vitro and in vivo, and compared to the potential of human stem cells from exfoliated deciduous teeth (SHEDs), also easily obtained. SHEDs are an adult mesenchymal stem cells (MSCs) used in bone bioengineering studies due to their osteogenic properties. We also performed in vivo lineage tracing to investigate the contribution of both cell types to new bone formation in a rat model of calvarial defect.

SHEDs and OOMSCs were cultured and characterized immunophenotypically (CD74, CD90, CD105, CD166, CD29 >95%; CD45, CD31 <3%). Both SHEDs and OOMDSCs cultures stained positive for alkaline phosphatase activity, a marker of osteoblast activity, 9 days after osteogenic induction (Invitrogen). At 14 days of induction SHEDs (85%), but not OOMSCs (0%), showed abundant extracellular matrix (ECM) mineralization. After 21 days, OOMSCs and SHEDs show equivalent mineralization of the ECM through quantification of alizarin red staining.

For in vivo evaluation SHEDs (n=5) and OOMDSCs (n=4) (10^6 cells) were added to a biphasic 80% porous scaffold (HA/β-TCP). Scaffolds were implanted into calvarial bilateral defects of Wistar rats, one scaffold empty and the other carrying MSCs. After 50 days of implantation, SHED induced bone formation was observed throughout the scaffold, giving a solid aspect to the region. Very few bone formation sites were observed in scaffolds associated to OOMSCs. Quantification of the new bone formed in the defects is in progress. A MicroCt scan 1176 (Skyscan) is used for quantification.

Immunohistochemical staining for human lamin A/C (Millipore) showed that bone cells (osteoblasts and osteocytes) were not from human origin. Human cells were observed in the connective tissue (CT) in the pores of the scaffold where bone tissue was not developed. The absence of human cells in the neo-formed bone at 50 days could be the result of bone remodeling occurred before that time. This hypothesis was discarded after an in vivo cell lineage tracing performed at 7, 15 and 30 days post-surgery using human lamin A/C staining that showed human cells localized only in the CT.

In conclusion, SHEDs differentiate into bone-forming osteoblasts in vitro faster than OOMSCs and enhance in vivo bone formation independently of their differentiation. This data suggests that SHEDs induce/enhance host response to the injury. Our results are in agreement with the newest literature that suggests an inductive role for MSCs in regenerative medicine. The comparison between 2 different cell types contribute to the better understanding of the role of each one in bone formation and shad light to the necessity of more comparative studies in vivo.

T-2247

SOX2+ CELLS ARE NECESSARY FOR SPINAL CORD REGENERATION IN XENOPUS

Rosana Muñoz, Dasfne Lee-Liu, Gabriela Edwards, Leonardo Almonacid, Mauricio Moreno, emilio Méndez, Víctor Tapia, Fernando Faunes, Francisco Melo, **Juan Larrain**
P. Universidad Católica de Chile, Santiago, Chile

Xenopus tadpoles are able to fully regenerate the spinal cord (SC) after injury, although this capacity is lost during metamorphosis. In our laboratory we are studying the cellular and molecular mechanisms that allow regeneration and how this capacity is lost during metamorphosis. In this meeting we will present results regarding two approaches that we have undertaken to advance this question: i) to evaluate the role of neural stem and progenitor cells (NPSC) and ii) high-through put analysis of the transcriptome activated in response to SC injury to identify the biological processes and genes required for regeneration.
To study the role of NPSC we have focus our studies on Sox2, a marker for NSPC. We have found that before metamorphosis ependymal cells lining the ependymal channel of the spinal cord express Sox2 and are actively proliferating. The number of Sox2+ cells and its proliferative capacity decrease, but does not disappear, during metamorphosis concomitantly with the lost of regenerative capacities.
Studies in regenerative tadpoles showed a rapid and transient activation of Sox2+ cells in response to SC injury (1 and 2 days post-injury, dpi). Between 6 to 10 dpi Sox2+ cells migrate and populate the ablation gap and restore continuity of the ependymal channel. Axon growth across the ablation gap starts at 10 dpi and almost normal continuity of the nerve tracts is restored after 20 days of regeneration. We have found that axon growth occurs in close contact to Sox2+ cells suggesting that these cells provide a permissive substrate for axon growth. Importantly, no activation of Sox2+ cells and no migration to the ablation gap occur in non-regenerative tadpoles.
The function of Sox2+ cells in regeneration has been studied by decreasing the levels of Sox2 by morpholino electroporation. We have found that reduction of Sox2+ diminishes regeneration of spinal cord (no restoration of swimming is observed). In conclusion Sox2+ cells are necessary for spinal cord regeneration in Xenopus. We are currently performing transplantation experiments to evaluate the ability of Sox2+ cells isolated from regenerative tadpoles to restore regeneration in post-metamorphic frogs.
To identify the biological processes and genes that are required for regeneration and that are missing from non-regenerative animals we have performed a high-throughput analysis of the SC transcriptome (RNA-Seq). We have found that approximately 5,700 transcripts respond differentially to injury in regenerative and non-regenerative stages. We have noted that regenerative tadpoles respond rapidly to injury and an important amount of changes in the transcriptome is already observed at 1 dpi, contrary to that the transcriptome of non-regenerative tadpoles only start to change at 6 dpi. Gene ontology analysis showed rapid activation in regenerative stages of cell cycle and metabolic process genes, contrary to that in non-regenerative stages immune system and stress response genes are mainly activated. These results suggest that rapid activation of proliferation of Sox2+ cells, permitted by activation of the proper metabolic circuits, is required for regeneration and an inadequate activation of the immune system and the stress response impairs activation of Sox2+ cells and is detrimental for regeneration.
We envision that this basic understanding should provide new insights in order to learn why mammalian spinal cord has very limited regenerative capacities and how this can be improved.

T-2248

FORELIMB FUNCTION AND TISSUE INTEGRITY OF THE INJURED SPINAL CORD IS IMPROVED FOLLOWING STEM CELL TRANSPLANTATION IN A NOVEL MODEL OF CERVICAL INJURY

Jared T. Wilcox, Kajana Satkunendrarajah, Jeff Zuccato, Yasmin Nasirzadeh, Farshad Nassiri, Michael G. Fehlings
Genetics and Development, University of Toronto, Toronto, ON, Canada

Spinal cord injury (SCI) to the cervical region represents the majority of clinical cases and a significant societal burden and personal impact due to loss of independence, employment and quality of life. Objective: Improve forelimb function and injured spinal tissue in a newly generated cervical SCI model through transplantation of adult and pluripotent-derived neural precursor cells (NPCs).
Methods: Cervical injuries were realized using calibrated clip contusion-compression in female Wistar rats at C6 vertebral level. Animals were randomized at 14 days post-injury into groups receiving: 1) adult brain-derived (a)NPCs, 2) embryonic stem cell-derived (ES-)NPCs (4x10^5 cells/animal); or, 3) vehicle-only control. All groups received intrathecal growth factors and minocycline and cyclosporine A to improve graft survival. Behavioural testing was conducted for 12 weeks post-injury for grip strength, Inclined Plane test, WARP contracture scale, BBB locomotor scale, and bladder recovery. Bi-directional axonal tracing and histological and immunohistochemical staining was performed at 4 and 12 weeks to quantitatively assess tissue preservation, cell engraftment and differentiation, motor neuron pools and neuroplasticity. Results: White and grey matter was significantly improved ($p>0.05$), with concomitant lesional volume and cavitation were significantly reduced ($p<0.05$) in

both aNPC and ES-NPC groups versus vehicle controls. Transplanted cells migrate to damaged tissue and deposit myelin. Forelimb function and grip strength is improved following cell engraftment at 6-12 weeks (373g vs 211g, $p<0.01$) compared to controls. Discussion: Transplantation of adult and ES-derived precursor cells in the injured cervical spinal cord results in significantly improved spinal tissue and forelimb function, warranting further work to improve this treatment paradigm for clinical translation.

T-2251

AGE-ASSOCIATED ALTERATIONS IN EPHRIN A1/EPHA2 SIGNALING INHIBIT MIGRATION OF HUMAN CARDIAC PROGENITOR CELLS

Polina Goichberg[1], Ramaswamy Kannappan[1], Fumihiro Sanada[1], Yingnan Bai[1], Sergio Signore[1], Maria Cimini[1], Federica del Monte[2], Jan Kajstura[1], Roberto Bolli[3], Marcello Rota[1], Piero Anversa[1], Annarosa Leri[1]

[1]Anesthesia and Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States, [2]CardioVascular Institute, Beth Israel Deaconess Medical Center, Boston, MA, United States, [3]Medicine, University of Louisville, Louisville, KY, United States

Effective regeneration of the myocardial tissue requires translocation of human cardiac progenitor cells (hCPCs) to the areas of damage. Signaling by Eph receptor tyrosine kinases and their ligands, ephrins, is widely implicated in controlling cell behavior during development, as well as in organ homeostasis and response to injury. We have established that in the adult heart, immature resident hCPCs preferentially express the EphA2 receptor, which binds to ephrin A1 ligand presented on the sarcolemma of the supporting cardiomyocytes. Down-regulation of the EphA2 protein abrogated hCPC adhesion to ephrin A1, and inhibited their motility. In an animal model of myocardial infarction, EphA2 activation with ephrin A1 facilitated trafficking of the transplanted hCPCs to the necrotic tissue, and led to the formation of new myocardium and improvement in cardiac function. We have further found that ephrin A1 level was elevated acutely after infarction in young mice. However, with physiological aging, a significant decrease in ephrin A1 protein was detected in senescent mouse and human cardiomyocytes. These data suggest that sub-optimal ephrin A1 expression hinders the trafficking of endogenous hCPCs in old hearts. Furthermore, cellular aging was characterized by a cell-autonomous defect in the migratory ability of hCPCs due to alterations in the EphA2 kinase function. Despite the comparable EphA2 expression in young and aged cells, EphA2 signaling was inhibited in senescent hCPCs. In old cells, EphA2 protein was subjected to post-translational modifications attributable to an accumulation of the reactive oxygen species. Oxidation of the EphA2 receptor interfered with ephrin A1-induced EphA2 auto-phosphorylation and compromised its ability to bind and activate Src family kinases (SFKs). In ephrin A1-stimulated young hCPCs, functional SFKs phosphorylated caveolin-1 protein and promoted caveolin-1-mediated EphA2 endocytosis. The endocytic vesicles containing activated EphA2 and SFKs were associated with EEA1-expressing early endosomes, signifying a signaling endosome formation in young hCPCs. The EphA2 signaling from early endosomes was required to sustain the motility-related responses in cells exposed to ephrin A1. In old hCPCs, a deficit in EphA2 activation altered its endocytosis, leading to receptor translocation to the lysosomal compartment and degradation. The insufficient function of the EphA2 receptor impaired the migration of old hCPCs in the infarcted myocardium. Based on the loss of the EphA2 signaling capacity in senescent cells, we have developed an approach to prospectively isolate hCPCs with a younger phenotype and preserved growth and regenerative reserve. In addition, we demonstrated that introduction of exogenous EphA2 ameliorated the ephrin A1 signaling capacity and increased the motility of the pool of aging hCPCs, which had not reached irreversible senescence. These results indicate that ephrin A1/EphA2-mediated pathway may serve as a therapeutic target for the treatment of the aging myopathy by facilitating the mobilization of resident or transplanted hCPCs.

T-2252

TISSUE REPAIR BY HEMATOPOIETIC STEM CELLS IN THE CASE OF A NON-HEMATOPOIETIC GENETIC DISORDER, CYSTINOSIS

Brian Yeagy, **Jay Sharma**, Swati Naphade, Celine Rocca, Sarah Ur, Stephanie Cherqui

Pediatrics, University of California, San Diego, La Jolla, CA, United States

Cystinosis is a lysosomal storage disorder which results from a genetic defect in the gene *CTNS* encoding the lysosomal cystine transporter protein, cystinosin. Cystine accumulates in every tissue compartment and leads to multi-organ failure. Our previous work has shown that transplantation of syngeneic wild-type (Wt) hematopoietic stem cell progeny (HSPC) in the mouse model of cystinosis, *Ctns*$^{-/-}$ mice, was effective in treating cystinosis. Bone marrow-derived cells integrated abundantly into all tissues leading to significant cystine decrease and tissue preservation.

The mechanisms under investigation for HSPC-based treatment are differentiation, trans-differentiation, cell fusion, cross correction, or a combination of these processes. We developed a new mouse model for cystinosis, the DsRed *Ctns*$^{-/-}$ mice, ubiquitously expressing the DsRed reporter gene. We then transplanted GFP-expressing HSPC so the differentiated bone marrow-derived cells (DsRed^{-}GFP^{+}) and the fused cells (DsRed^{+}GFP^{+}) could be unequivocally recognized, quantified and sorted. Using confocal microscopy, flow cytometry and DNA array analysis, we showed that stem cells mainly differentiate in tissue-resident phagocytic cells (kupffer cells in the liver, microglia cells in the brain, and dendritic cells in the kidney). We also showed that they could play a role in tissue repair by fusing with or by phagocytosing the apoptotic host cells. Using a lentiviral vector driving the expression of the fusion protein cystinosin-eGFP, we also showed that cystinosin could be transferred from CTNS-expressing cells to Ctns-deficient adjacent cells *in vitro* and *in vivo*.

We optimized *in vitro* functional assays involving macrophages to study these two potential mechanisms in the context of cystinosis. We showed that macrophage-mediated phagocytosis was stimulated in the presence of Ctns$^{-/-}$ fibroblasts and that transfer of cystinosin-eGFP was performed via nanotube-like structures extending from the macrophages to cystinotic cells.

T-2253

REGENERATION OF EXPERIMENTALLY-PRODUCED CARTILAGE DEFECTS IN OVINE KNEE JOINTS USING AUTOLOGOUS BONE MARROW AND ADIPOSE TISSUE DERIVED MSCS AND CHONDROCYTES

leila Taghiyar renani[1], Mohamadreza Baghaban Eslaminejad[1], Mohamadmehdi Dehghan[1], farhad Taghizade[2], Nesa Fani[1]

[1]*Department of Stem Cell and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, Tehran, Iran, Islamic Republic of,* [2]*Faculty of Veterinary Medicine University of Tehran, Tehran University, Tehran, Iran, Islamic Republic of*

Introduction: Osteoarthritis is a progressive disorder of the joints caused by gradual loss of articular cartilage; witch naturally possesses a limited regenerative capacity. Autologous chondrocyte implantation (ACI) could be one approach to regenerate an articular cartilage defect, but first-generation ACI has limitations. Mesenchymal stem cells (MSCs) are another alternative that can be used to regenerate articular cartilage defects. MSCs were first isolated from bone marrow samples. In addition investigations have indicated that adipose tissue contains MSC-like cells. In this study we compared cartilage regenerative capability of these cells and autologous chondrocytes in experimentally-created chondroal defects at ovine model.

Method: In this study 8 male sheep were studied. All transplantation was performed autologously. Marrow MSCs were isolated from tibia and adipose stem cells were isolated from the animal tail adipose tissue. Articular chondrocytes were prepared from cartilage biopsy taken from the animal ribs using an enzymatic digestion. About 2×10^6 cells from each sources were labeled with PKH and incorporated into collagen I gels to produce 3-D constructs. Full-thickness chondral defects were then created at lateral and medial femoral condyles of animal knee joints using pouch. The defects were transplanted with constructs prepared from three different cells and covered with periosteom taken from the same animal tibia. Implantation of collagen without cells was taken as the control. About 18 weeks after transplantation, the animals were sacrificed and the joint was opened. Articular surface was observed at implantation site and scored in terms of surface smoothness. Repair tissue was then removed, histologically pre-

pared and further evaluated in terms of integration of implanted construct into host tissue. In addition the kind of cartilage tissue (hyaline or fibro) was determined for each group.
Results: According to our scoring, there was no significant difference regarding the smoothness of articular cartilage surface in experimental groups. In contrast, at control, the surface was observed to be rough. At fluorescence microscopy, PKH-labeled cells were obvious. Regarding the thickness of repair tissue, the thickness tended to be significantly higher at either marrow or adipose MSC than chondrocyte groups ($P<0.05$). In this regard, there was no significant difference among marrow MSC and adipose stem cells. The lowest thickness was observed at collagen without cell group. Similarly, there was better integration observed at both stem cell groups compared with either chondrocyte group or collagen without cells. Moreover, chondrocye/collagen construct possessed better integration than collagen without cells. Interestingly, stem cell/collagen constructs produced hyaline-like constructs, while at 2 out of 8 chondrocyte/collagen group, bone formation was observed. Implantation of collagen without cells resulted in fibrocartilage formation. No hypertrophic chondrocytes were observed in stem cell groups but in chondrocyte group, there were some hypertrophied cells.
Conclusion: In conclusion, either marrow MSCs or stem cells from adipose tissue are appropriate cell candidate for cell-based treatment of cartilage defects. Since the repair resulted from both tissue was comparable, adipose tissue could be presented as suitable alternative to marrow MSCs**.**

T-2254

HUMAN PLURIPOTENT STEM CELL-DERIVED NEURAL PRECURSORS IMPROVE STROKE OUTCOME AND REDUCE SECONDARY DEGENERATION IN THE RECIPIENT BRAIN

Seong Mi Choi[1], Dae-Sung Kim[1], Eun Hae Kim[2], Sung-Rae Cho[3], Dong-Wook Kim[1], Han-Soo Kim[2]
[A]Physiology, [B]Laboratory Medicine, [C]Rehabilitation Medicine, [1]Yunsei Univ Coll of Medicine, Seoul, Korea, Republic of

In the present study, we compared the therapeutic effects of human embryonic stem cell-derived PSA-NCAM$^+$ neural precursor cells (NPC$^{PSA\text{-}NCAM+}$) with human bone marrow-derived mesenchymal stem cells (MSCs) on brain infarction area and neurological outcomes in stroke animal models. NPC$^{PSA\text{-}NCAM+}$ or MSCs (1×10^6 cells) were transplanted into rat ipsilateral striatum 2 days after permanent middle cerebral artery occlusion (pMCAo). When brain tissues were harvested on day 24 post-transplantation, infarction areas were significantly reduced in NPC$^{PSA\text{-}NCAM+}$- and MSC-transplanted groups compared to that of control. The motor and reflex performance identified that NPC$^{PSA\text{-}NCAM+}$ administration showed remarkable functional recovery as early as 3 day post-transplantation, while MSCs showed a modest effect. Immunohistochemistry showed that grafted hNu-positive NPC$^{PSA\text{-}NCAM+}$ survived for at least 24 days post-transplantation without tumor formation, whereas only a few grafted MSCs were detected. Most of the hNu-positive NPC$^{PSA\text{-}NCAM+}$ also expressed early migrating neuronal markers which include DCX and Tuj1, and only a fraction of hNu-positive cells differentiated into Map2$^+$ mature neurons. Expression of ED-1 and GFAP was lower in NPC$^{PSA\text{-}NCAM+}$-transplanted brains compared to that of MSC-treated groups implying that NSC$^{PSA\text{-}NCAM+}$ suppressed inflammatory responses in the ischemic brain. In addition, NPC$^{PSA\text{-}NCAM+}$ enhanced endogenous angiogenesis disclosed by α-SMA$^+$ immunoreactivity in the lesion, more than angiogenesis induced by transplanted MSCs. Taken together, our findings suggest that early paracrine effects exerted by NPC$^{PSA\text{-}NCAM+}$ have much greater impact on the course of functional improvement as underlined therapeutic mechanism. *This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MEST) (2012M3A9C7050126, 2012M3A9B4028639 and 2010-0020408).*

T-2255

SEQUENTIAL REPROGRAMMING AND RE-DIFFERENTIATION OF REACTIVE ASTROCYTES TO IDENTIFY DOWNSTREAM TARGETS OF JAGGED1/NOTCH1 SIGNALING IN THE PERI-INFARCT AREA AFTER STROKE.

Matthew D. Lecomte, Andrea L. Bibeau, Jeffrey L. Spees
Medicine, University of Vermont, Burlington, VT, United States

Early after stroke, reactive astrocytes reduce injury by regulating blood brain barrier integrity, immune cell invasion, and neuronal survival. Previously, we reported that a specialized subpopulation of RC2-positive reactive astrocytes in the peri-infarct area, directly adjacent to the stroke infarct core, are regulated by Notch1 signaling after stroke. The RC2-positive reactive astrocytes may provide important clues regarding therapeutic targets to reduce stroke injury or improve stroke outcome. Although RC2-positive reactive astrocytes proliferate in vivo after stroke, they do so poorly in culture. To provide robust cell numbers for screening, we isolated proliferating reactive astrocytes from the peri-infarct area 3 days after stroke and reprogrammed them into reactive astrocyte-derived neural stem cells (Rad-NSCs). We found that Rad-NSCs provided a useful system to expand large numbers of progenitor cells that could then be re-differentiated back into RC2-positive reactive astrocytes. After 1 week of differentiation, we seeded re-differentiated RC2-positive reactive astrocytes on laminin-coated plates in the presence or absence of immobilized Jagged1 (Notch1 ligand). After 48 hrs, we lifted the cells, isolated Total RNA and ran Affymetrix microarrays to determine Jagged1-induced gene expression. In response to immobilized Jagged1, the re-differentiated reactive astrocytes upregulated mRNAs for Hes1, Hes5, and Hey2, transcriptional targets of NICD1; this result confirmed successful Notch1 (NICD1)-based signaling. In addition, we observed increased transcription of mRNAs for a variety of secreted proteins involved in blood brain barrier integrity and repair, regulation of inflammation, glutamate metabolism and uptake, and angiogenesis. To verify our screen, immunohistochemistry was conducted on brain tissue from mice 3 days after stroke induced by permanent distal middle cerebral artery occlusion. Based on our gene expression data for control and Jagged1-stimulated reactive astrocytes, we stained for Beta-dystroglycan, an astrocytic transmembrane protein known to stabilize the blood brain barrier (endothelial basement membrane). Notably, Beta-dystroglycan localized to the bodies and end-feet of reactive astrocytes, but was especially concentrated in the end-feet of reactive astrocytes associated with the microvasculature in the peri-infarct area. Our results suggest that the Rad-NSC re-differentiation system may provide a useful tool to model signaling after injury with the aim of identifying novel targets for treatment of stroke and perhaps other CNS injuries.

T-2256

SUBSTANCE-P, A STEM CELL MOBILIZER, REDUCES PROGRESSION OF HEPATIC FIBROSIS IN TAA-LEPTIN INDUCED-LIVER CIRROSIS MODEL

Suna Kim, Hyun Sook Hong, Eunah Lee, Youngsook Son
Genetic engineering, Kyung Hee University, Yong In, Korea, Republic of

Liver cirrhosis is characterized by fibrosis and scar accumulation. The transfusion of bone marrow derived stem cell is an effective therapy to substitute a drug or liver transplantation. However, current stem cell therapy confronts several problems such as high cost, long duration of ex vivo culture and low engraftment in vivo. Interestingly, our group reported that substance-p (SP) recalls endogenous wound healing mechanism by mobilizing bone marrow stem cells [Hong et al., Nature Medicine, 2009]. Furthermore, we also reported SP plays a role as an anti-inflammatory modulation in spinal cord injury [Jiang MH et al., Neuroreport, 2012]. In this study, we explored that SP may have a therapeutic potency alleviating liver cirrhosis, which is induced by thioacetamide (TAA) and leptin. Liver cirrhosis was induced by intra-peritoneal injection of TAA (200 μg/g, BW) plus leptin (1 μg/g, BW) three times a week for 6 weeks. Successful development of liver cirrhosis was confirmed at 6 weeks using histological analysis. To evaluate the effect of SP on chronic liver fibrosis, SP (5 nmole/kg, BW) was intravenously injected to liver cirrhosis mice three times a week from 6 weeks to 10 weeks, accompanying by injection of TAA and leptin, simultaneously. In control group, saline was intravenously injected to liver cirrhosis mice on the same way as SP.
In result, at 10 weeks, SP decreased the formation of hepatic micronodule on liver external surface. Compared with control group, higher alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level were observed in SP-injected group. It means that SP may retard development of chronic liver cirrhosis induced by TAA and leptin because ALT/AST level is lower in chronic liver injury than acute injury. And based on histological analysis, SP-injected group showed decrease in the number of infiltrated immune cells, comparing with control group. Moreover,

SP administration decreased collagen deposition and portal-portal septum formation surrounding hepatic lobule. In order to elucidate whether SP may have a role in prevention of fibrosis, we carried out immunostaining with α-SMA, which is a representative marker for activated myofibroblast, namely hepatic stellate cell. As a result, the number of activated myofibroblast was decreased in SP-treated group. Aimed toward clarifying the mechanism for hepato-protective potency of SP in environment of acute and chronic hepatic injury, experiments such as cytokine analysis, western blot and BrdU staining are on the way. From these data, SP may be one of the promising candidates to prevent progression of hepatic fibrosis.

T-2257

UNDERSTANDING CELLULAR DYNAMICS OF TISSUE REPAIR BY LIVE IMAGING

Kailin Mesa, Panteleimon Rompolas, Valentina Greco
Genetics, Yale University, New Haven, CT, United States

Tissue injury can lead to both destructive as well as regenerative processes. However the complex balance between these two dynamic processes is not fully understood. Our lab has recently pioneered a technique for in vivo, time-lapse imaging, which allows us to map for the first time the dynamic behaviors of stem cells within the skin hair follicle over time in a live mouse (Rompolas, Nature 2012). Using mouse skin tissue injury models, in combination with live imaging, we have a temporal approach to investigate the integrated tissue repair responses including cell death, proliferation and migration. Additionally, we are utilizing lineage tracing approaches to determine by live imaging which cellular compartments help to fuel this repair process.
Therefore, by the use of two photon live imaging our lab has gained new insights on the dynamic relationship between tissue destruction and repair, which has broad implications for the treatment of trauma as well as neoplastic and degenerative diseases.

T-2258

SAFETY AND EFFICACY OF ALLOGENEIC UMBILICAL CORD BLOOD THERAPY FOR CHILDREN WITH CEREBRAL PALSY

MinYoung Kim[1], Kyunghoon Min[1], Ki Baik Hahm[2]
[A]Rehabilitation Medicine, [B]Internal Medicine, [1]CHA Bundang Medical Center, Seongnam-si, Korea, Republic of

1. Background:
Cerebral palsy (CP) is a group of neurodevelopmental conditions with abnormal movement and posture resulted from non-progressive cerebral dysturbances. Cell therapy has been investigated to alleviate the neurologic impairment. Umbilical cord blood (UCB) is known to reduce sensorimotor and/or cognitive dysfunction in animal models of CP. Autologous UCB is known to have therapeutic benefits. In case of lack of autologous UCB, allogeneic UCB can be an alternative.
2. Methods:
1) Patients
Patients between 6 months and 20 years old were eligible with a diagnosis of CP. This study was designed as a double blinded randomized trial (clinicaltrials.gov NCT01528436).

UCB with ≤ 2 Human Leukocyte Antigen (HLA) dysparities and ≥ $2x10^7$ total nucleated cells(TNC)/kg were selected. Cyclosporine and solumedrol 3mg/kg were used.
2) Outcomes
Primary outcomes are changes in Gross Motor Function Measure (GMFM) and Gross Motor Performance Measure (GMPM). Seconday outcomes are changes in Korean version of Bayley Scales of Infant Development-II (K-BSID-II), Functional Independence Measure for Children (WeeFIM), manual muscle testing (MMT), Pediatric Evaluation of Disability Inventory (PEDI). Those outcomes were assessed 3 times at baseline, 4 weeks and 12 weeks.

^{18}F-FDG PET was acquired before and two weeks after UCB administration.
3. Results

1) Participants: From February 2012 to July 2012, 41 patients were screened. 35 patients completed the study. UCB group received allogeneic UCB administration. 16 of 18 UCB group received UCB through IA and 2 patients via IV.

2) Outcomes

UCB group and control group were not different in general charicteristics. There was a trend of improved changes in GMFM and GMPM in UCB group compared with Control group without statistical sinificance.

In our previous clinical trial, UCB was more effective at 6 months. Hence, the results of 6 months after UCB administration were compared as an extended analysis. Changes in GMPM at the same interval showed significant improvement in UCB group (P =0.008).

In UCB group, changes in BSID-II motor raw score, WeeFIM, MMT were significantly higher in HLA 1 mismatched than in HLA 2 mismatched (Ps <0.04).

As ^{18}F-FDG PET study, in UCB group, bilateral occipital and temporal lobes showed decreased glucose metabolism.

There was a correlation in TNC and changes in K-BSID-II Motor raw score between baseline and 3 months in UCB group (rho=0.54, P=0.02).

Blood samples were obtained and association of functinal changes and levels of various biomarkers were analysed. Increment of IL-8 was correlated with changes in K-BSID-II Motor raw score. Changes in 'Biomarker A' was correlated with GMFM changes. Changes in 'Biomarker A' was also correlated with changes in IL-10.

There were no serious adverse events during the study period.

4. Conclusion:

Additive strategy was suggested to be required to augment the efficacy of UCB therapy or more extended period would be needed than 3 months. Next, more compatible UCB is expected to bring out better outcomes. Plus, higher cell dose is said to be related with better outcome. In PET study, glucose metabolism of periventricular areas was prominently declined in UCB group. One of characteristic MRI findings in CP was periventricular leukomalacia (PVL), whicn was though to be affected by inflammatory insults. Hence, UCB might play a certain role to decrease the glucse metabolism of the areas in UCB group through suppressing inflammation.

1

T-2261

CELL THERAPY FOR RADIATION-INDUCED ORAL MUCOSITIS WITH BONE MARROW-DERIVED CELLS

Takashi I[1], Yoshinori Sumita[1], Tokutaro Minamizato[1], Takayoshi Tobita[1], Younan Liu[2], Simon D. Tran[2], Izumi Asahina[1]

[1]Department of Regenerative Oral Surgery, Nagasaki Univecity Graduate School of Biomadecal Sciences, Nagasaki, Japan, [2]Faculty of Dentistry, McGill University, Montreal, QC, Canada

[Background]Oral mucositis is a serious and painful side effect for patients with head and neck cancer who received radiation-and/or chemo-therapy. It causes not only the limitation on the ability of patients to tolerate cancer therapies in some cases but also the harmful effects on the quality of life. However, current clinical strategies cannot efficiently prevent the occurrence of oral mucositis. Therefore, effective therapy should be developed urgently. Recently, we have shown that cell therapy with bone marrow derived cells (BMDCs) displays the preventive effects to the radiation-induced salivary gland hypofunction (*Sumita et al. Int J Biochem Cell Biol 2011*). Therefore, transplanted BMDCs also may affect the occurrence of oral mucositis preclusively after head and neck cancer therapy since BMDCs comprise the various stem/progenitor cells in high density. **[Objective]**To investigate whether exogenous BMDCs can prevent or rescue the radiogenic tongue mucositis in a mouse model.**[Methods]**BMDCs isolated from male C3H mice were transplanted to the female mice just after the irradiation through the tail vein. After 2, 5, 7 and 11 days of irradiation, the tongues were harvested, and the macroscopic, histological and immunological analyses were performed to clarify the effects of BMDC transplantation.**[Result]**After 2 days of irradiation, the tongues of non-treated mice (IR) showed significant apoptosis of the cells throughout the basal cell layer while few cells were observed in that of BMDCs-treated mice (BM). Contrarily, the expression of stem cell markers (c-Kit and Sca-1) was detectable in the samples of BM. After 7 days of irradiation, while the ulcer area became maximum in the tongues of IR, the area of ulcers

formed in BM specimens was approximately 60% of IR and preserved the epithelial thickness. Consistent with this appearance, histological analysis shows that tongues of BM demonstrated an increased level of tissue regenerative activity such as blood vessel formation and cell proliferation. Meanwhile, we detected PKH26-expressed cells, which were derived from transplanted BMDCs, in the connective tissues right under the basal cell layer. Finally, after 11 days of irradiation, the area of ulcers in BM decreased remarkably compared with that in IR.**[Conclusion]**Our data suggests that cell therapy with BMDCs can prevent the maximum ulceration caused by head and neck irradiation. We are currently investigating the additional therapeutic effects and mechanisms of BMDC therapy to the radiation-induced oral mucositis.

T-2262

A STEM CELL BASED APPROACH TO TREATING MULTIPLE SCLEROSIS USING DRUG LIKE SMALL MOLECULES

Chelsea Green, Luke Lairson
The Scripps Research Institute, San Diego, CA, United States

Oligodendrocytes, the glia that mylinate nerve axons of the central nervous system, are regenerated throughout adulthood. Widespread populations of oligodendrocyte precursor cells (OPCs) migrate to sites of demylination, and proceed to mature into oligodendrocytes. The progressive phases of multiple sclerosis (MS) are associated with inhibited differentiation of the OPC adult stem cell population. Remission in MS is largely dependent on OPCs migrating to sites of demylination and subsequently differentiating into mature oligodendrocytes. This study focuses on controlling stem cell fate and developmental potential through the use of small molecules. Using a high content imaging cell-based assay, we have screened a diverse collection of over 80,000 drug-like small molecules in order to identify compounds that are directly responsible for stimulating the process of remylination. OPCs were treated with compound and stained for myelin basic protein. They were then screened using fluorescent microscopy and the intensities of fluorescence analyzed in GeneData. Thyroid hormone was used as a positive control. Compounds within four standard deviations of intensity of the positive control were characterized as hits. A secondary confirmation screen was then conducted in triplicate, as well as a counter screen to filter out compounds that were initially fluorescent. Compounds that passed this second screen were then tested under a dose response, in which cells were treated with eight doses of compound. The compounds that induced differentiation at greater than 5uM concentration were characterized as hits. The structures of these compounds were analyzed and several molecular scaffolds that consistently appear as hits have been identified. Representative compounds from the scaffold groups are currently being selected and will be analyzed with Western Blotting and RT-PCR to further characterize their ability to induce OPC differentiation in functioning oligodendrocytes.

T-2263

MSC-TRANSPLANTATION IN SKELETAL MUSCLE INJURY LEADS TO FUNCTIONAL IMPROVEMENT AND IS ACCOMPANIED BY A SLOW TO FAST FIBER TYPE SHIFT

Tobias Winkler[1], Philipp von Roth[1], Piotr Radojewski[1], Georg N. Duda[2], Carsten Perka[3]
[A]Center for Musculoskeletal Surgery, Berlin-Brandenburg Center for Regenerative Therapies, [B]Berlin-Brandenburg Center for Regenerative Therapies, BSRT, Julius Wolff Institute, [C]Center for Musculoskeletal Surgery, Berlin-Brandenburg Center for Regenerative Therapies, BSRT, [1]Charité Universitaetsmedizin Berlin, Berlin, Germany

Objectives: Functional deficits after traumatic or iatrogenic skeletal muscle injuries cannot be addressed by current treatment options. We could demonstrate in previous experiments that a local transplantation (TX) of mesenchymal stem cells (MSCs) is efficient in the regeneration of contraction forces after trauma. However, the mechanisms behind the effect of the cells still have to be analyzed. The aim of the present study was to look into muscle fiber composition changes following MSC-TX after a selective skeletal muscle injury.
Methods: An open crush trauma of the left soleus muscle was performed in 20 male Sprague Dawley rats. 2.5x10^6 autologous MSCs, which were cultivated from tibial biopsies, were transplanted into the injured muscles of 10 an-

imals 7 days after trauma (group 1, n=10). An injection of saline solution was used as a control (group 2, n=10). 28 days after trauma we performed an in vivo muscle force measurement and analyzed the muscles histologically for the development of fibrosis and the distribution of fiber types.
Results: Muscle contraction forces were significantly improved by MSC-TX. (Ratio injured/uninjured soleus muscle: fast-twitch forces untreated: 0.45 (0.32-0.73), treated: 0.76 (0.51-1.15), p=0.01; tetanic forces untreated: 0.34 (0.16-0.48), treated: 0.63 (0.4-1.21), p=0.04). In the histological analysis we found no differences in the amount of collagenous fibrotic tissue (untreated vs. treated, p=0.42). MSC-TX led to a shift of muscle fiber types from slow towards fast MHC positive fibers (untreated vs. treated, p=0.007).
Conclusion: In the presented experimental study an effect of locally transplanted MSCs for the treatment of skeletal muscle injuries could be shown on a structural level. For the first time a shift of muscle fiber types towards fast MHC fibers after MSC-TX could be demonstrated. These results indicate a possible new mode of action of MSC-TX in skeletal muscle injuries.

Tissue Engineering

T-2265

NOVEL MECHANISMS OF VEGF ACTION DURING NEURAL REGENERATION IN ISCHEMIC SKELETAL MUSCLE

Dmitry Shvartsman
School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, United States

Severe neuropathies result from traumatic injuries or the sudden loss of tissue perfusion (e.g. ischemic stroke), and current treatment options typically do not achieve complete regeneration of the damaged nerve. Vascular endothelial growth factor (VEGF), a potent angiogenic factor, also plays a number of roles in the nervous system. Here, we show that sustained and localized VEGF delivery from hydrogels ameliorates loss of skeletal muscle innervation after injury by slowing degeneration and promoting re-growth of damaged axons. Nerve growth factor (NGF) and glial derived neurotrophic factor (GDNF) mediated VEGF-induced axonal regeneration, and both are induced by VEGF presentation. Surprisingly, the activity of these neurotrophic factors regulates VEGF-driven angiogenesis via control over endothelial cell sprouting. Altogether, these studies produce evidence of novel mechanisms of VEGF action, further broaden the understanding of angiogenesis and axonal regeneration, and suggest approaches to improve axonal and ischemic tissue repair therapies.

T-2266

RESTORATION OF LIVER FUNCTION BY TRANSPLANTATION OF TISSUE-ENGINEERED HEPATIC-LIKE TISSUES

Oscar Lee[1], **Wei-Cheng Jiang**[2]
[A]Institute of Clinical Medicine, [B]Institute of Biomedical Engineering, [1]National Yang-Ming University, Taipei, Taiwan

Liver transplantation has been the only effective treatment for severe hepatic failure up to the present. However, a limited supply of
organs has restricted the application of this approach. The creation of innovative substitutes for transplantation has thus become an imperative. In this study, we utilized decellularization technique to generate the acellular liver scaffolds (ALS) that preserve the native biochemical compounds and the innate physical structures of inherent livers. These results show that hepatic differentiation from mesenchymal stem cells (MSCs) within the ALS is superior to culturing the cells in two-dimensional environments *in vitro*. Moreover, the hepatic-like tissues (HLT) that were generated from the reconstituted MSCs within the ALS were able to act as functional grafts and rescue acute hepatic failure, which then contributed to liver regeneration after transplantation *in vivo*. These findings suggest that HLT have high therapeutic potential when used for hepatic tissue engineering treatment of liver failure.

T-2267

ADIPOSE DERIVED CELL-FREE EXTRACT PROTECTS MICE AGAINST HINDLIMB ISCHEMIA

Jeong-Min Kim, Dong Yeon Lee, Keun-Hwa Jung, Kon Chu, Soon-Tae Lee, Jae-Joon Ban, Jungsuk Ryu, Ji-Young Huh, Jangsup Moon, Manho Kim, Sang Kun Lee, Jae-Kyu Roh
Seoul National University Hospital, Seoul, Korea, Republic of

Background: Stem cell transplantation has been considered as promising therapeutic strategy in various disease models, but its clinical application has been delayed due to potential safety issues concerning malignancy formation and immunologic reaction. Adipose stem cells are known to secrete multiple growth factors and cytoprotective cytokines. In this study we investigated whether adipose derived stem cell lysate (ASCL) could attenuate tissue damage in mice ischemic hindlimb model.
Methods: Human adipose derived stem cells were harvested and ASCL was extracted by mechanical dissolution followed by centrifugation to collect supernatant. In vitro angiogenic potential was evaluated with HUVEC by Matrigel tube forming assay and migration assay. Ischemic hindlimb model was produced with BALB/C nude mice by double ligation and cutting of femoral artery. Human ASCL was administered by intramuscular injection for five days after induction of ischemic hindlimb. Lower extremity circulation was monitored by laser Doppler perfusion image on 5th and 15th day, and necrotic area and blood vessel density were evaluated 15 days after ischemia.
Results: Treatment with ASCL enhanced migration and tube forming capacity of HUVEC in vitro. Ischemic hindlimb model showed significant perfusion decrease compared to the other normal side, which was attenuated by ASCL treatment (56.0±4.3% versus 77.1±5.3%). The treatment of ASCL limited necrotic area (40.0±13.6% versus 15.7±5.5%) with maintenance of vascular area on 15th day after ischemia.
Conclusions: This study demonstrated that adipose derived cell extract harbors a strong tissue protective effect after ischemic insult with restored local circulation in hindlimb ischemia model. Stem cell based treatment paradigm could be extended to the manipulation of cell derived tissue protective material.

T-2268

THE ISOLATION AND DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED STEM CELLS USING MEMBRANE FILTRATION

Akon Higuchi[1], Akon Higuchi[2]
[1]*Department of Chemical Engineering and Material Science, National Central University, Jhong-li, Taiwan,* [2]*Department of Reproduction, National Research Institute for Child Health and Development, Tokyo, Japan*

Stem cells are an attractive source of cells for tissue engineering and cell therapy because of their unique biological properties. There are major ethical concerns regarding the use of embryonic stem cells (ESCs) in potential human therapies. However, human adipose-derived stem cells (hADSCs) hold promise for regenerative medicine, particularly for cell therapies and tissue engineering applications. hADSCs can be isolated from liposuction-derived adipose tissue by centrifugation followed by cultivation in cell culture dishes for at least one passage. The cultivation of cells derived from adipose tissue is necessary to purify hADSCs (i.e., “the culture method” for the purification of hADSCs) because the adipose tissue also contains adipose cells and other cell types. Purifying hADSCs via the culture process requires 5-12 days. If hADSCs could be purified from adipose tissue in a shorter time period (i.e., less than 30 min) using a cell purification device such as that used in the membrane filtration method, cell therapy and tissue engineering applications using autologous hADSCs might become more efficient. In this study, hADSCs were purified from a suspension of human adipose tissue cells (stromal vascular fraction) by the conventional culture method and by membrane filtration through polyurethane (PU) foam membranes. PU membranes with a pore size of 11 μm were selected for the purification of hADSCs by the membrane filtration method because the isolated hADSCs were determined to be approximately 10 μm in size based on microscopic observation. hADSCs can be obtained from a suspension of human adipose tissue cells using the membrane filtration method in less than 30 min, whereas the conventional culture method requires 5-12 days. hADSCs that express the mesenchymal stem cell markers CD44, CD73, and CD90 were concentrated in the recovery solution from the PU membranes; no hADSCs were isolated in the permeate. After filtration, the cells expressing the mesenchymal stem cell markers were 3-4.5 times more concentrated than in the initial suspension of human adipose tissue cells, with the level of concentration depending on the surface modification of the PU membrane. Cells expressing the stem cell-associated marker

CD34 could be successfully isolated in the recovery solutions, whereas CD34+ cells could not be purified by the conventional culture method. The hADSCs in the recovery solution demonstrated a superior capacity for osteogenic differentiation than did the cells in the suspension of human adipose tissue cells where the osteogenic differentiation was evaluated by alizarin red staining (calcium deposition) and von Kossa staining (calcium phosphate deposition) as well as osteogenesis-related gene expression of osteocalcin and osteopontin. These results suggested that the hADSCs with the capability for osteogenic differentiation adhered to the PU membranes. Linear correlations between the expression rates of CD44, CD73, and CD90 and the osteogenic differentiation ability were identified (r=0.854-0.964). Cells expressing higher levels of the mesenchymal stem cell-specific surface markers were shown to have a greater capacity to differentiate along the osteogenic lineage.

T-2271

PERIURETHRAL INJECTION OF AUTOLOGOUS ADIPOSE-DERIVED REGENERATIVE CELLS FOR THE TREATMENT OF INTRACTABLE STRESS URINARY INCONTINENCE: OUTCOME OF A PRELIMINARY CLINICAL TRIAL

Yamamoto Tokunori[1], Suzuki Satoshi[2], Funahash Yasuhito[1], Matsukawa Yoshihisa[1], Gotoh Momokazu[1]
[A]Urology, [B]clinical regeneration, [1]Univesity of Nagoya, Nagoya, Japan

Introduction and Objective: We report a novel cell therapy technique using autologous adipose-derived regenerative cells (ADRCs) (Yamamoto T IJU 2012) for intractable stress urinary incontinence caused by urethral sphincter deficiency and the early outcomes .
Materials and Methods: 11 cases with persistent intaractble stress urinary incontinence more than 1 year after total prostatectomy(n=9) and transurethral resection of prostate(n=2) were performed. After liposuction of 250 mL of adipose tissue from the abdomen, we isolated ADRCs from this tissue using the CelutionTM system. Subsequently, the isolated ADRCs and a mixture of stem cells and adipose tissue were transurethrally injected into the rhabdosphincter and submucosal space of the urethra, respectively. Outcomes during a 1-year follow-up were assessed by a 24-hour pad test (mean leakage amount per day during 4 consecutive days in a 24-hour pad test), a validated patient questionnaire (ICIQ-SF), urethral pressure profile, contrast-enhanced transrectal ultrasonography, and MRI. The present study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine and by the Japanese Ministry of Health, Labour and Welfare, and written informed consent was obtained from the patients.
Results: After injection, urinary incontinence progressively improved during the 1 year follow-up in all patients, i.e., decreased leakage volume in a 24-hour pad test. The mean leakage amount per day in patient group(n=5) with moderate incontinence more than 200ml/day and patient group(n=6) with mild incontinence less than 200ml/day decreased by 29%(from 531.1ml to 379.9ml) and 48%(from 85.6ml to51.9ml) respectively . In the urethral pressure profile, both maximum urethral closing pressure and functional profile length increased in both patients. In all patients, ICIQ-SF demonstrated a progressive improvement in frequency and amount of incontinence and quality of life during the 3-month follow-up. Ultrasonography and magnetic resonance imaging demonstrated sustained presence of the injected adipose tissue. Enhanced ultrasonography revealed a progressive increase in blood flow to the injected area. No significant adverse events were observed peri- and postoperatively.
Conclusion: This preliminary study demonstrated that periurethral injection of autologous ADRCs is a safe and feasible treatment modality for patients with intractable stress urinary incontinence caused by urethral sphincter deficiency.

T-2272

THE THERAPEUTIC POTENTIAL OF SPHERE-FORMING CELLS FROM THE HUMAN SALIVARY GLAND

Sarah Pringle[1], Marianne van der Zwaag[2], Djoke van Gosliga[1], Monique A. Stokman[3], Ronald van Os[4], Robert P. Coppes[1]
[A]Cell Biology and Radiation Oncology, [B]Cell Biology, [C]Radiation Oncology and Oral Maxillofacial Surgery, [D]European Research Institute Into Biology of Ageing, [1]University Medical Center Groningen, 9713 AV, Netherlands

Hyposalivation and its consequence xerostomia are sequelae of salivary gland (SG) functional ablation, commonly caused by radiotherapy (RT) treatment for head and neck cancers. Xerostomic patients are unable to speak, swallow, eat or sleep without difficulties, and will be plagued by such symptoms for the rest of their lives. In mice, we showed previously that transplantation of cells isolated from salispheres cultured from SGs, rescued irradiated mice from hyposalivation. We aim to characterize human salispheres as a possible therapeutic avenue for the treatment of RT-induced hyposalivation in humans.

Our murine salisphere isolation protocol could be effectively translated for use with human SG tissue. Human salispheres cells (hS cells) were able to self-renew for ≥ 5 passages *in vitro*, with a peak proliferative ability around passage 3 (sphere-forming capacity 3.83 ± 0.99 %; population doublings 3.26, ± 0.75). After 12 days in an *in vitro* differentiation assay, single cell-derived human salispheres generated organoids containing duct- and acinar cell-like structures. These organoids contained at least two distinct cells types, namely cytokeratin^{+} (Cyt^{+}) and aquaporin-5^{+} (AQP5^{+}). Expression of Cyt and AQP5 is associated with ducts and acinar cells of adult human SGs, respectively.

In order to investigate the *in vivo* therapeutic potential of hS cells, we irradiated the SG of immune-deficient mice. After xenotransplantation into SGs in this mouse model, hS cells proliferate extensively, as inferred by dilution of the PKH26 membrane label and co-immunostaining for human nuclei. Transplanted hS cell foci contained duct-like structures, where Cyt^{+}, AQP5^{+} and α-amylase^{+} (a further marker of human SGs acinar cells) cells could be detected.

In a functional, clinically relevant read-out of the *in vivo* abilities of hS cells, saliva flow of mice transplanted with 50,000 cells per SG was monitored over a 3 month time course. 55 % (10/18) of mice transplanted with 50,000 hS cells demonstrated saliva flow ≥ 2 SDs greater than that of the control group ('responder animals'). In post-transplant salisphere cultures, responder animals also demonstrated significant greater salisphere counts of which qPCR analysis revealed that 1.36 ± 1.03 % of cells were of human origin. Human cells were not detected in non-responder post-transplant cultures. Finally, western blot analysis of saliva from responder animals demonstrated secretion of human SG protein Muc5B into transplanted mouse saliva. These *in vitro*, functional *in vivo* and *ex vivo* assays provide compelling evidence that hS cells are not only capable of proliferation and differentiation *in vitro*, but can integrate into transplanted SG, rescue saliva production, and secrete human SG protein.

We believe hS cell transplantation represents a logical, longer term option for the management of radiation-induced xerostomia. We propose also that the observed effects of hS transplantation are due to the regenerative potential of human SG stem or progenitor cells, which remain to be fully defined. We are currently working on the translation of our hS knowledge towards GMP standards, and optimization of our cell delivery technique, and the ultimate realization of a hS cell therapy for xerostomia.

T-2273

APPLICATION OF HYPERDRY AMNIOTIC MEMBRANE PATCHES OVER THE DEFECT LESION OF MUCOSAL MEMBRANE IN ORAL AND NASAL CAVITY

Michiro Fujisaka[1], Hideo Shojaku[1], Hirohiko Tachino[1], Motonori Okabe[1], Toshio Nikaido[2]

[A]otolaryngology, [B]regenerative medicine, [1]University of Toyama, toyama, Japan

Human amniotic membrane (AM) is a tissue of fetal origin that consists of three major layers: a single epithelial layer, a thick basement membrane, and an avascular mesenchyme. Because AM has many favorable characteristics including being non-antigenic, adhering well to wounds, being large enough to cover wounds such as large burns, promoting epithelization and healing, and decreasing inflammation, it has been widely used clinically for membranous allografts in the fields of dermatology and ophthalmology. Recently, the efficacy of the AM graft used as a substitute for an autograft, i.e., as a temporalis fascia graft, was reported as well in the field of otology. Preserved hyperdry AM graft patches were attached over the bony surface of the mastoid cavity after canal wall down tympanoplasty. The time to complete epithelization offered by the AM was significantly faster than that by the temporal fascia.

AM has high pliability and adherence capability. The aim of the present study was to determine whether it is possible to cover the defect lesion of mucosal membrane in oral and nasal cavity.

In the application of AM for oral cavity, we used to the patient with oral mucosal squamous cell carcinoma. After removal of the malignant tissue, it was happened the defect of mucosal membrane. The defect lesion of mucosal membrane was covered with AM and tied over with gauze. After 3 days from operation, gauze was removed and so AM had attached to the defected lesion. The surface of wound was clear and there was no fur. In comparison with the wound by tonsillectomy, there was no fur, significantly faster of the time to complete epithelization. The wound by tonsillectomy takes usually about 1 week to disappear fur and 2 weeks to complete epithelization. But the wound covered with AM had no fur after operation and the time to complete epithelization is faster after removal gauze.
In the application of AM for nasal cavity, we used to the patient with hamartoma on the nasal septm. Hamartoma was removed by nasal endoscopic surgery. After removal the tumor, it happened the defect of nasal septum mucosal membrane and naked septum bone. In the nasal cavity, naked bone usually produce the crust for long time after operation. It is daily problem for the patient. But the wound covered with AM produced no crust and attached faster. We concluded that it was possible that AM is available for the operation in oral and nasal cavity.

T-2274

BONE MARROW ASPIRATE CONCENTRATE PROMOTE THE BONE ENGINEERING WITH rhBMP-2

Mayumi Umebayashi[1], Keiichiro miura[1], Yoshinori Sumita[1], Yuya Nakatani[1], Kazuhiro Nagai[1], Weijian Zhong[2], Izumi Asahina[1]
[1]Nagasaki Univ, Nagasaki City, Japan, [2]Dalian Medical University, Dalian, China

[Background] The stem/progenitor cells derived from bone-marrow are the most useful autologous cells for the bone regeneration. Our recent data demonstrated that human bone-marrow aspirate concentrate (BMAC) can provide the new bone tissue on the same level as the amount as peripheral-blood PRP-induced bone tissue (*Zhong et al. PLoS One 2012*). However, BMAC comprises not only concentrated platelets but also stem/progenitor cells in high density. Therefore, BMAC may display the highly osteoenhancive ability synergistically when applying it for the bone engineering with BMP-2 protein. Utilization of BMAC may bypass the time-consuming and technically difficult process of cell expansion and differentiation, enabling both harvesting and transplanting of BMAC during the same surgical procedure. **[Objective]** To evaluate the synergistic effects produced by human BMAC and recombinant human (rh) BMP-2 on promoting the ectopic bone formation in an immunodeficient mouse model. **[Methods]** Bone-marrow and peripheral-blood aspirates from human volunteers were concentrated by Megellan automated blood separator. After cell counting, six groups of nude mice ([BM]; bone-marrow aspirate, [BMAC]; BM-concentrate, [BM-PPP]; BM-platelet poor plasma, [PB]; peripheral-blood aspirate, [PRP]; PB-platelet rich plasma, [PB-PPP]; PB-platelet poor plasma) were transplanted with graft materials subcutaneously. β-TCP particles which absorbed rhBMP-2 were employed as scaffold and the samples were harvested 2, 4, and 8 weeks after transplantation. **[Results]** BMAC and PB enhanced the rhBMP-2-induced bone formation compared with others, but these two groups showed the no significant differences regarding the rate of new bone area. Meanwhile, the significant bone-marrow formation in BMAC groups was recognized compared with that in PRP groups at 4 weeks after transplantation. **[Conclusion]** Our data suggests that the synergistic effect of human BMAC and rhBMP-2 gives highly osteoinductive properties to β-TCP particles *in vivo*. We are currently investigating the additional osteoenhancive ability of BMAC.

T-2275

NON INVASIVE SOURCES OF ADULT STEM CELLS TO BE USED IN BONE TISSUE ENGINEERING FOR CLEFT LIP AND PALATE PATIENTS

Daniela F. Bueno[1], Daniela Tanikawa[3], Bruno Pimenta[4], Marilia T. Martins[5], Gerson S. Kobayashi[4], Meire Aguena[4], Tatiana Jazedze[2], Cassio Eduardo Raposo-Amaral[6], Maria Rita Passos Bueno[4]

[A]Stem Cell Biology, [B]Genetic and Evolutive Biology, [1]Sirio Libanes Hospital and Sao Paulo University, Sao Paulo, Brazil, [A]Plastic Surgery, [B]Genetic and Evolutive Biology, [C]Oral pathology, [2]Sao Paulo University, Sao Paulo, Brazil, [3]Plastic Surgery, SOBRAPAR hospital, Campinas, Brazil

Cleft lip and palate (CLP), one of the most frequent congenital malformations, affects the alveolar bone in the great majority of the cases, and the reconstruction of this defect still represents a challenge in the rehabilitation of these patients. The gold standard in alveolar bone reconstruction is autogenous bone grafts. However, these surgical procedures may be subjected to complications such as donor area morbidity, post-surgical reabsorption and infections. To circumvent these problems, researchers have been focusing on the development of bone tissue engineering strategies and osteogenic substances that may offer alternative methods with minimal or no donor site morbidity for bone grafts. Therefore, in order to identify a non-invasive alternative source of stem cells with osteogenic potential without conferring morbidity to the donor, we have used orbicular oris muscle (OOM) and levator palatine muscle (LPM) fragments , which are regularly discarded during surgery repair (cheiloplasty and palatoplasty) of CLP patients, to make bone tissue engineering. We also used dental pulp (DP)obtained from deciduous teeth of CLP patients to make bone tissue engineering. We obtained cells from OOM, LP and DP fragments, of CLP patients using previously described pre-plating technique. These cells, through flow cytometry analysis, were mainly positively marked for five mesenchymal stem cell antigens (CD29, CD90, CD105, CD73,CD166), while negative for hematopoietic (CD45) and endothelial cell marker(CD31). After induction under appropriate cell culture conditions, these cells obtained from OOM,LPM and DP were capable to undergo chondrogenic, adipogenic, osteogenic, and skeletal muscle cell differentiation, as evidenced by immunohistochemistry. We also demonstrated that these cells together with a collagen membrane lead to bone tissue reconstruction in a critical-size cranial defects previously induced in non-immunocompromised rats. The presence of human DNA in the new bone was confirmed by PCR with human specific primers and immunohistochemistry with human nuclei antibodies. In conclusion, we showed that cells from OOM, LPM and DP have phenotypic and behavior characteristics similar to other adult stem cells, both in vitro and in vivo. Our findings suggest that all these tissues (OOM, LPM, and DP) represent a promising source of stem cells for alveolar bone grafting treatment (bone tissue engineering), particularly in young CLP patients.

T-2276

IMPROVED CHONDROCYTE DEDIFFERENTIATION THROUGH THE USE OF MICROTISSUES AND HYPOXIA

Betul Kul, Peter Levett, Parisa Ghanavi, William Lott, Travis Klein, Justin Cooper-White, Ross Crawford, **Michael R. Doran**
Translational Research Institute, Queensland University of Technology, Brisbane, Australia

INTRODUCTION: A major limitation in cartilage tissue engineering therapies is that chondrocytes dedifferentiate during *ex vivo* expansion on 2-dimensional surfaces. It is common practice to aggregate the expanded cells into 3-dimensional pellets, in the presence of induction factors, to facilitate their redifferentiation. Pellets are generally formed from $1x10^5$-$5x10^5$ chondrocytes, and these macropellets have diameters ranging from 1-2 mm. The diffusion of metabolites over the 1-2 mm pellet length-scales is inefficient, resulting in radial tissue heterogeneity. Herein we describe how such diffusion gradients, and tissue heterogeneity, can be overcome by forming hundreds of smaller diameter micropellets of 166 cells each, rather single macropellets of 200,000 chondrocytes.
METHODS: Through this study we developed a cost effective microwell fabrication strategy, and this microwell platform was used to manufacture hundreds of micropellets. The microwell platform, which is an array of 360x360 µm microwells was cast into polydimethylsiloxane (PDMS). It was then surface modified, to be non-adherent, with an electrostatic multilayer of hyaluronic acid and chitosan to enhance micropellet formation. This surface modification prevented cell attachment and spreading on the PDMS surface.
RESULTS: In micropellets the production of sulfated glycosaminoglycan (sGAG) was significantly greater than in macropellet cultures. Collagen II gene expression in micropellets was greater than in macropellets, and histological staining indicated that the matrix was more uniform. These characteristics were further enhanced when micropellet cultures were maintained in a hypoxic atmosphere (2% O_2). Using an optimized micropellet redifferentiation

methodology, we manufactured hundreds of micropellets and then assembled them into larger cartilage tissues. Our results indicate that micropellets amalgamate efficiently, forming virtually seamless macro tissues.
DISCUSSION: Here we describe a method for microwell fabrication and micropellet manufacture. Chondrocyte redifferentiation is enhanced in hypoxic micropellets relative to traditional macropellet cultures. These micropellets can be assembled into larger tissues.

T-2277

CD133+ HEMATOPOIETIC STEM CELLS ARE SUFFICIENT TO IMPROVE REGENERATION IN BIOLOGICAL IMPAIRED BONE HEALING OF AGED

Anke Dienelt[1], Andrea Sass[2], Bernd Preininger[3], Katharina Schmidt-Bleek[2], Georg N. Duda[1]
[A]BCRT and Julius Wolff Institut, Center for Musculoskeletal Surgery, [B]Julius Wolff Institut, Center for Musculoskeletal Surgery, [C]Center for Musculoskeletal Surgery, [1]Charité - Universitätsmedizin Berlin, Berlin, Germany

Clinical improvements in bone regeneration appear mandatory specifically for aged patients. Thus strategies to overcome delayed healing or non-union scenarios (affecting 5-10% patients) are needed. Several in vitro studies reported that CD133+ hematopoietic stem cells bare angiogenic capacities and contribute to a better outcome concerning ischemia induced angiogenesis in vivo. A local administration of these specific cells to a fracture site in an intra-operative setting appears feasible as a new treatment option for biological impaired fracture healing. We hypothesize that the local administration of CD133+ cells provides sufficient angiogenic and osteogenic stimulus to enhance healing in aged individuals.
We analyzed availability, angiogenic and osteogenic properties of human CD133+ cells derived from healthy young and aged, male and female probands (n=8) in vitro to answer the question whether cells obtained from aged patients bare the same regenerative potential as cells from young donors. For this purpose flow cytometric analyses, co-cultures with endothelial cells and osteogenic differentiation assays together with mesenchymal stroma cells were performed.
Finally, the regenerative capacities of CD133+ cells were validated in vivo in an aged animal model with biological impaired fracture healing. Rat CD133+ cells were extracted from donor animals and transplanted locally to a 2 mm osteotomy gap. Bone healing was investigated after 2, 4 and 6 weeks by µCT and histological analysis.
The experiments confirmed that human CD133+ cells bare high angiogenic capacities, even though these properties decline with aging. Additionally, human CD133+ cells had no negative effect on osteogenic differentiation, when isolated from aged people. The regenerative properties of CD133+ cells derived from aged donors could also be proven in vivo by an enhanced bone tissue formation after local CD133+ cell transplantation. This improved bone healing was accompanied by a 2fold increase in bone mineral content and went along with a 3fold elevated development of new blood vessels within the fracture site.
Aiming to identify a new source of cells utilizable for cell therapy in aged people, we could document that CD133+ hematopoetic stem cells feature even in aged donors sufficient bone regenerative capacities. Thus, an application of these cells to fracture sites appears a promising strategy for the treatment of biological impaired fracture healing.

T-2278

FUNCTIONALITY AND DURABILITY OF ENGINEERED HUMAN VASCULAR NETWORKS FROM ENDOTHELIAL PROGENITOR CELLS IN A DEEP THERMAL WOUND.

Donny Hanjaya-Putra[1], Jason A. Burdick[2], Charles Steenbergen[3], Sharon Gerecht[4]
[1]Surgery, BIDMC - Harvard Medical School, Boston, MA, United States, [2]Bioengineering, University of Pennsylvania, Philadelphia, PA, United States, [A]Pathology, [B]Chemical and Biomolecular Engineering, [3]The Johns Hopkins University, Baltimore, MD, United States

Successful regeneration of many injured and diseased tissues requires timely vascularization to support the renewal and differentiation of new tissues. Thermal injuries caused by third-degree burns heal slowly due to damage of both epidermal and dermal layers, as well as trauma to the underlying tissues. A promising approach is the transplantation of fully functionalized microvascular networks from autologous endothelial colony-forming cells (ECFCs) that can rapidly integrate with the circulatory system. Understanding the survival, functionality and integration of engineered human microvasculature are crucial aspects in determining the healing outcome of the thermal wound. In this study, we utilized tunable hyaluronic acid (HA) hydrogels to generate robust vascular networks by ECFCs and assessed their functionality and durability in a deep third-degree burn wound model. We demonstrate that using material design we can control ECFCs vascular morphogenesis in synthetic matrices, as a clinically relevant alternative to natural materials. Vacuole and lumen formation are RGD dose-dependent and are recognized by the ECFCs through integrin $\alpha_5\beta_1$ and $\alpha_V\beta_3$ subunits. Integration of MMP-sensitive-peptide to the HA hydrogels enabled ECFCs to sprout, branch, and form complex vascular networks. To determine the regenerative capabilities of the human engineered vasculature, we tested their functionality and durability in a third-degree burn wound model. Two days after the initiation of thermal wound, matured and stabilized vascular constructs were transplanted into the site of injury. On day 3 following transplantation, macrophages rapidly degraded the hydrogels during a period of inflammation and proliferation; the host's vasculatures infiltrated the construct, connecting with the human vessels within the wound area. The growth of mouse vessels near the wound area supported further integration with the implanted human vasculatures. During this period, the majority of the vessels (~60%) in the treated wound area were human. Although no increase in the density of human vessels was detected during the proliferative phase, they temporarily increased in size and peaked at day 7. As the wound reached the remodeling period during the second week after transplantation, the vasculatures including the transplanted human vessels generally regressed and few microvessels, wrapped by mouse smooth muscle cells and with a vessel area less than 200 μm^2 (including the human ones) remained in the healed wound. Collectively, we show that ECFCs vascular morphogenesis can be regulated in a synthetic matrix, resulting in a robust and functional microvasculature, which facilitate the healing process of a deep thermal wound. This study offers useful insights for the development of vascularization strategies for wound healing and ischemic conditions, for tissue-engineered constructs, and for stem cell vascular biology.

T-2281

GENERATION OF FUNCTIONAL DURABLE ENGINEERED BLOOD VESSELS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS.

REKHA SAMUEL[1], LAURENCE DAHERON[3], SHAN LIAO[1], TRUPTI VARDAM[1], WALID S. KAMOUN[1], ANA BATISTA[1], CHRISTA BUECKER[3], XIAOXING HAN[1], PATRICK AU[1], DAVID T. SCADDEN[2], DAN G. DUDA[1], DAI FUKUMURA[1], RAKESH K. JAIN[1]

[A]RADIATION ONCOLOGY, [B]CENTER FOR REGENERATIVE MEDICINE, [1]MASSACHUSETTS GENERAL HOSPITAL, BOSTON, MA, United States, [2]STEM CELL AND REGENERATIVE BIOLOGY, HARVARD STEM CELL INSTITUTE, BOSTON, MA, United States

A critical challenge of human induced pluripotent stem (hiPS) cell-based regenerative medicine is the efficient generation of potent vasculogenic cells. Biologically relevant systems to assess functionality of the engineered vessels in vivo are equally important for such development.
Methods: We report a novel approach for the derivation of endothelial precursor cells from hiPS cells using triple combination of selection markers: CD34, neuropilin-1 (NP-1) and KDR, and an efficient 2-D culture system for hiPS cell-derived endothelial precursor cell expansion. Functionality of blood vessels in cranial window models of Severe Combined Immunodeficient (SCID) mice was determined by non-invasive longitudinal in vivo Multiphoton Laser Scanning Microscopy to parameters that included red blood cell (RBC) velocity, blood flow and permeability to bovine serum albumin.
Results: We successfully generated endothelial cells from hiPS cells obtained from healthy donors and formed stable functional blood vessels in vivo - lasting for 280 days in SCID mice. The RBC velocities of engineered blood vessels were comparable to normal endogenous host vessels (1.36+/- 0.3mm/s), and demonstrated a higher per-

meability as compared to endogenous vessels. In addition, we developed an approach to generate mesenchymal precursor cells (MPCs) from hiPS cells in parallel that exhibited trilineage adipocytic, chondrocytic and osteoblastic differentiation comparable with human bone marrow derived mesenchymal stem cells. Moreover, we successfully generated functional blood vessels in vivo using these endothelial cells and mesenchymal precursor cells derived from the same hiPS cell line. These data provide proof-of-the-principle that autologous hiPS-cell-derived vascular precursors could be used for in vivo applications, once safety and immunological issues of hiPS-based cellular therapy have been resolved. Additionally, the durability of hiPS-derived blood vessels in vivo demonstrates a potential translation of this approach in long-term vascularization for tissue engineering and treatment of vascular diseases. Finally, the hiPS-derived vasculogenic cells may be an abundant source to examine vascular defects in a dish in diseases such as Diabetes where access to target tissue such as kidney or retina is challenging.

T-2282

PURIFICATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURAL PROGENITORS FOR REGENERATIVE MEDICINE APPLICATIONS

Gonçalo M.C. Rodrigues, Andreia S. Matos, **Tiago G. Fernandes**, Maria Margarida Diogo, Joaquim M.S. Cabral
Department of Bioengineering, Instituto Superior Técnico, Lisbon, Portugal

Human induced pluripotent stem cells (hiPSCs) are a promising source of cells for clinical applications due to their ability to self-renew and to differentiate into cells from the three embryonic germ layers. One of the major hurdles related to the use of PSC-derived populations in Regenerative Medicine applications is the presence of PSC that are unable to differentiate since these cells have the potential to generate tumors after being transplanted and may interfere with the outcome of the *in vitro* differentiation protocol. One strategy to tackle this problem is to make the depletion of these “contaminating” cells during the differentiation process. Thus, this work aimed to embrace and overcome the challenge of producing a hiPSC-derived neural progenitor (NP) population that can be potentially used for the treatment of neurodegenerative diseases, through establishment of a neural commitment method under chemically-defined conditions based on the use of recombinant morphogens/growth factors followed by the depletion of the remaining PSCs using magnetic activated cell sorting (MACS). The differentiation of hiPSCs into NPs was obtained by means of the dual-SMAD inhibition protocol, based on the use of two small molecules, SB431542 and LDN193189, which was found to be a very robust and cost-effective method. This method resulted in the production of a slightly heterogeneous Pax6- and Nestin-positive NP population with 80% efficiency. The kinetics of the neural commitment process of the model hiPSC line was studied and the average specific growth rate and doubling times of these populations were estimated as μ=0.45±0.11 day^{-1} and T_d=36.97±9.04 h, respectively. By comparing these values with the ones obtained during the expansion of hiPSC, μ=0.72±0.15 day^{-1} and T_d=23.1±4.8 h, these results suggest a decrease in the number of cell divisions after the initiation of the neural commitment protocol.
After performing the neural commitment procedure, a magnetic activated cell sorting (MACS) method was used for purification of the heterogeneous cell population using anti-Tra-1-60 micro beads for negative selection of the unwanted PSCs. Mathematical models were developed to predict the final cell purity and the purification yield after the MACS-based purification procedure according to the percentage of Tra-1-60-positive cells present in the initial cell population. This method was found to be efficient if the initial cell-populations contained less than 30% of “contaminant” cells. When the percentage of cells to deplete was higher, the performance of this method was significantly reduced. However, since the hiPSC-derived populations differentiated through the double SMAD inhibition method consistently yielded an average of 20% PSCs, the results obtained showed a very good outcome, with purified samples containing only approximately 2% of Tra-1-60 positive cells. The MACS methodology thus seems to be appropriated for the initial stages of the separation of tumorigenic PSC from a population of human neural progenitors. This work paves the way for the establishment of an integrated process for the production and purification human neural progenitors for Regenerative Medicine applications.

T-2283

DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS INTO MOTOR NEURONS ON ELECTROSPUN FIBER SCAFFOLDS

Xizi Dai, Yen-Chih Huang
Biomedical Engineering, Florida International Universitiy, Miami, FL, United States

Conventional therapies for repairing peripheral nerve injuries are based on either autografts or allografts. These repair procedures can often yield superior neural functional recovery but still tend to be impaired by some drawbacks. For instance, the result of autografting often comes with size mismatch between the recipient and donor nerves, loss of function at the donor site, and the need for multiple surgeries. For allografts, patients are exposed to the risk of immune/disease-related complications. Tissue engineering has emerged as an excellent approach for the regeneration of damaged tissue, with the potential to circumvent the limitations of autologous and allogenic tissue repair. In addition, pluripotent stem cells become attractive cell sources for tissue engineering applications, since they are able to self-renew and differentiate into any cell types of three germ layers including motor neurons. In this study, we are interested in examining the feasibility of differentiating mouse embryonic stem cells (ECSs) into motor neurons on three dimensional electrospun fiber scaffolds. These scaffolds fabricated to mimic the native ECM components have the potential to be used for bridging the injured nerve transection in neural tissue engineering. The goal of this study is to differentiate ESCs into functional motor neurons on biodegradable poly (glycerol-dodecanoate) (PGD) and its derivatives poly (glycerol dodecanoate co-fumarate) (PGDF) and poly (glycerol dodecanoate co-itaconate) (PGDI) fiber scaffolds. Firstly, the differentiation process was carried out on tissue culture dishes. Noggin and fibroblast growth factors were used to enhance the neuroectoderm differentiation. When neural stem cells appeared, the cells were harvested and seeded on the 3D fiber scaffolds. Incubating with retinoic acid and sonic hedgehog, the cells were induced to motor neuron. In order to monitor the cells on each differentiation stage, RT-PCR and immmunohistochemistry were used to characterize these cells. The differentiated neurons expressed motor neuron specific markers such as HB9 and Isl1. In addition, some cells were also shown to differentiate into glia cells on the scaffolds, which were positively staining with GFAP. In summary, the preliminary results demonstrated that the electrospun scaffolds can support the mouse ESCs survive and differentiate into motor neurons. Ongoing experiments are currently underway to further examine the electrophysiology of the motor neurons on the scaffolds.

T-2284

SELF RENEWING PODOCYTE PROGENITORS FROM AMNIOTIC FLUID

Stefano Da Sacco[1], Kevin V. Lemley[1], Sargis Sedrakyan[1], Ilenia Zanusso[2], Astgik Petrosyan[1], Janos Peti-Peterdi[3], James Burford[3], Rezvan Habibian[4], Roger De Filippo[1], Laura Perin[1]
[1]Children's Hospital Los Angeles, Los Angeles, CA, United States, [2]University of Padova, Padova, Italy, [3]University of Southern California, Los Angeles, CA, United States, [4]Integrated Genetics, Monrovia, CA, United States

Most renal pathologies leading to End Stage Renal Disease (ESRD) originate within the glomerulus and when podocytes are lost, there is only a limited possibility to replace these cells, as they are functionally post-mitotic cells with very limited proliferative capacity to regenerate themselves. In addition, it is very challenging to isolate human podocytes and culture them without coming across their de-differentiation and thus loosing most of the peculiar traits characteristic of these highly specialized cells. Amniotic fluid is in continuity with multiple developing organ systems, including the kidney. Committed, but still stem-like cells from these organs may thus appear within the amniotic fluid. We report having established for the first time a stem-like cell population derived from amniotic fluid (AKPC-P) and possessing characteristics of podocyte precursors.
These cells can be propagated for many passages and differentiated toward a podocyte phenotype. We compared these cells with conditionally immortalized podocytes, the current gold standard for in vitro studies. After in vitro differentiation, both cell lines have similar expression of the major podocyte proteins and functional properties. In particular, AKPC-P acquired the typical podocyte like traits with a markedly arborized appearance and increased expression of various podocyte markers including WT-1, nephrin and podocin. Most importantly, AKPC-P showed

the ability to produce the mature collagen IV alpha 3-4-5 chains along with ILK, CD151 and integrins, essential for cell-matrix interaction. When seeded onto decellularized glomerular tufts AKPC-P engrafted and formed foot-like processes. In addition, AKPC-P were able to produce VEGF and showed to respond to both angiotensin II and flufenamic acid (a TRPC6 calcium channel agonist) by increasing calcium intake and cell contraction. AKPC-P rearranged their cytoskeleton and lost primary processes after administration of nephrotoxic agent puromycin aminonucleoside. In contrast to immortalized cells, this cell population has a more nearly normal cell cycle regulation and a clear developmental pattern of specific protein expression. These cells can be easily isolated from the amniotic fluid of humans or mammalian model organisms, including animal models of genetic diseases such as Alport syndrome. Their characteristic developmental pattern of expression of several podocyte proteins suggest that, in addition to studying gene regulation, extracellular matrix production and target drug development, hAKPC-P could represent the first in vitro model that can be used for analysis of podocyte development.

T-2285

BIOMATERIAL-BASED EXPANSION OF CORD BLOOD-DERIVED HEMATOPOIETIC STEM CELLS

Mónica S. Ventura Ferreira[1], Willi Jahnen-Dechent[1], Norina Labude[2], Thomas Hieronymus[3], Martin Zenke[3], Rebekka K. Schneider[2], Sabine Neuss[1]

[A]Helmholtz Institute for Biomedical Engineering, Biointerface Group, [B]Institute of Pathology, [C]Helmholtz Institute for Biomedical Engineering, Cell Biology, [1]RWTH Aachen University, Aachen, Germany

BIOMATERIAL-BASED EXPANSION OF CORD BLOOD-DERIVED HEMATOPOIETIC STEM CELLS

Mónica S. Ventura Ferreira[1,2], Willi Jahnen-Dechent[2], Norina Labude[1], Manfred Bovi[3], Thomas Hieronymus[4], Martin Zenke[4], Rebekka K. Schneider[1], Sabine Neuss[1,2]

[1] Institute of Pathology, RWTH Aachen University, Aachen, Germany

[2] Helmholtz Institute for Biomedical Engineering, Biointerface Group, RWTH Aachen University, Aachen, Germany

[3] Electron Microscopic Facility, University Hospital Aachen, Aachen, Germany

[4] Institute for Biomedical Engineering, Department of Cell Biology, RWTH Aachen University, Aachen, Germany

More than 20,000 cord blood (CB) transplants were performed worldwide since the first CB transplant was done around 20 years ago. Using CB for hematopoietic stem cell (HSC) transplantation is particular interesting. However, the limited number of HSC obtained from one single CB unit impairs clinical applicability of CB transplantation to adult subjects. We are now developing an efficient *ex vivo* strategy for HSC expansion. In order to better mimic the native bone marrow (BM) microenvironment, we follow a multifactorial strategy including the use of three-dimensional (3D) scaffolds, cytokine supplementation and stromal support. In our study, we assessed (i) the expansion efficiency of different cytokine combinations for maximal CB-CD34+ cell expansion, (ii) basic compatibility of sixteen different biomaterials produced as 2D substrates for CB-HSC growth and selected a group of six for further testing, (iii) four different three dimensional biomaterial scaffolds in combination with strong cytokine supplementation in presence/absence of umbilical cord (UC) blood-derived mesenchymal stem cells (MSC) for CB-CD34+ cell expansion. Methods for analysis included CFSE cell proliferation, cell toxicity, cell differentiation, cell migration, clonogenic potential, scanning electron microscopy and *in vivo* transplantations (mouse model). Results show (1) greater CB-HSC expansion using a combination of cytokines composed of SCF, TPO, FGF-1, Angptl-5 and IGFBP2 (STFAI), compared to other combinations, (2) PCL, Resomer® RG503 and fibrin supplemented with STFAI support CB-CD34+ cell expansion in 2D culture formats, (3) UC-MSC support stimulates CB-HSC expansion. Our optimized expansion strategy includes the use of 3D fibrin scaffolds and UC-MSC support in presence of STFAI for superior CB-HSC proliferation, differentiation, adhesion, migration and homing efficiency. For deep understanding of the mechanisms behind the functional success of our strategy, molecular analyses are ongoing. Real time RT-PCR is used to evaluate relative gene expression in 3D fibrin CB-HSC cultures for a group of genes of interest. In order to assess extracellular matrix remodelling on 3D fibrin scaffolds for CB-HSC expansion, protein expression is assessed using a panel of immunohistochemical markers.

T-2286

ANALYSIS OF TISSUE REACTIONS IN RATS AFTER IMPLANT OF DIFFERENT COMPOSITIONS OF NANOTECHNOLOGICAL SCAFFOLDS FOR TISSUE ENGINEERING

Gerson Xavier Acasigua[1], Daniela Steffens[2], Joelson Tomedi[3], Tattiana Enrich Essvein[4], Juliana Figueiredo Faria[4], Vinicius Coelho Carrard[5], Anna Christina Medeiros Fossati[5], **Patricia Pranke**[6]

[1]Both authors have the same importance in this work; Post-graduate Program in Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [2]Both authors have the same importance in this work; Hematology and Stem Cell Laboratory, Faculty of Pharmacy, Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [3]Post-graduate Program in Physiology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [4]Faculty of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [5]Department of Morphological Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [6]Hematology and Stem Cell Laboratory, Faculty of Pharmacy; Post-graduate Program in Physiology - Federal University of Rio Grande do Sul, Stem Cell Research Institute, Porto Alegre, Brazil

Congenital malformations and loss of specialized tissues represent a challenge to reconstructive surgery and tissue engineering offers new possibilities of treatment for these conditions. It comprises of the utilization of materials, cellular therapy and biomolecules to create new ways of producing tissues ex vivo for implantation. In this process, materials are used to build biocompatible and biodegradable scaffolds, which simulate the natural extracellular matrix. Therefore, one of the main qualities of a material is to prevent significant inflammatory reactions when introduced in the body. Thus, this work aims to verify the intrinsic inflammatory response to scaffolds produced with different compositions of natural and synthetic materials by the electrospinning technique. A total of 12 Wistar rats were divided into four experimental periods (7, 14, 30, and 60 days). The inflammatory reactions were evaluated in five scaffold groups: group 1, Poly (lactic-co-glycolic acid) PLGA; group 2, Collagen with hialuronic acid (9:1) (w:w); group 3, Poly (DL-lactic acid) PDLLA; group 4, PDLLA with the microalga Spirulina incorporated in a concentration (wt/wt) of 2% (PDLLA/Sp); and group 5, control (empty tube). The animals were anesthetized and five 0.5 cm long incisions were made in the animals' backs. Polyethylene tubes, approximately 10 mm long and 1.5 mm in internal diameter, were used to implant the scaffolds in the animals. At the end of each experimental period, an excisional biopsy of the implant area was performed with a safety margin of 1 cm. The resulting specimens were fixed in formalin and set in paraffin blocks. Sections were cut along the axis of each tube, mounted on slides and stained with hematoxylin and eosin. The slices were examined under an optical microscope by a calibrated examiner. The slides were analyzed qualitatively for the following cellular inflammatory components: presence of neutrophils, lymphocytes/plasmocytes, eosinophils and macrophages/giant cells. Cellular events were classified according to the following scale: (1) absent; (2) mild; (3) moderate; and (4) intense. Fiber condensation was classified as follows: (1) absence of collagen fibers, (2) presence of a thin layer of collagen fibers, and (3) presence of a thick layer of collagen fibers. The presence or absent of abscess was also verified and it was characterized by collections of active neutrophils in necrosis process. The partial results, obtained through the histological analysis from the scaffolds implanted in 12 rats, did not show a statistically significant difference among the materials used. This find is probably due to the fact that the number of samples necessary for the study has not yet been achieved. However, there was a statistical tendency for increasing inflammatory responses in the PDLLA/Sp scaffolds (neutrophils p=0.05, and lymphocytes/plasmocytes p=0.09). The presence of abscess also showed a tendency of increase in group 4 (p=0.07). The fibroplasias, presence of eosinophils, macrophages/giant cells and lymphocytes/plasmocytes did not show statistical difference between all the groups. In order, therefore, to confirm these results, 12 more animals are being analyzed to increase the sample size. However, the partial results already analyzed indicate that the scaffolds, excepting group 4, appear to present adequate tissue response when implanted in the connective tissue of rats and can therefore be considered an useful tool for the construction of biomaterials.

T-2287

ESOPHAGEAL TISSUE ENGINEERING IN A LARGE ANIMAL USING A FLOW BIOREACTOR

Panagiotis Maghsoudlou[1], Lucy Papaioannou[1], Luca Urbani[1], Ryan Dee[2], Maria Menikou[2], Stavros Loukogeorgakis[1], Panicos Shangaris[1], Daniel Ditchfield[1], Alexander Seifalian[2], Simon Eaton[1], Paolo De Coppi[1]
[A]Institute of Child Health and Great Ormond Street Hospital, [B]Centre for Nanotechnology & Regenerative Medicine, [1]University College London, London, United Kingdom

Aim of the study: Tissue engineering of the oesophagus (TEO) has been proposed as a therapeutic alternative to oesophageal substitution. Acellular matrices are ideal for TEO because they contain all the extracellular matrix (ECM) information. Here we aim to establish a methodology for creating a scaffold in a large animal model and assessing different seeding techniques to repopulate the muscle layer.
Methods: We decellularized rabbit oesophagi (n=12) with different cycles of detergent-enzymatic treatment (DET) and evaluated the preservation of the ECM and acellularity of the scaffold by histology, immunofluoresence, SEM/TEM, biomechanical testing, ECM component quantification and CAM assay. To repopulate the scaffold, C2C12 cells were seeded in either static or under-flow conditions using a bioreactor. 1000000 cells / cm2 were seeded inside and outside the scaffold and allowed to adhere. Static and under-flow seeded scaffold were analysed with histological examination after 24 and 72 hours.
Main results: Efficient decellularization was achieved after 3 cycles of DET as evidenced by histology and DNA quantification, with a significant decrease in respect to the fresh tissue (1.07 vs 0.21 µg/mg wet tissue, $p<0.05$). Collagen, elastin and glycosaminoglycans levels were preserved following decellularization. EM showed the preservation of the oesophageal strata. The acellular scaffold allowed the adhesion of C2C12 cells in both the seeding conditions. However, using a bioreactor we obtained a better and more homogeneous distribution of cells, as highlighted with histology.
Conclusion: Developing of an acellular scaffold for oesophageal tissue engineering that preserves the ECM components and architecture of the original tissue is essential for therapy. The use of dynamic seeding methodologies allows improved seeding on the scaffold.

T-2288

ENGINEERING REPRODUCIBLE NEURAL TISSUE FROM INDUCED PLURIPOTENT STEM CELLS

Stephanie Willerth[1], Amy Montgomery[2], Alixandra Wong[1], Lin Sun[1]
[A]Medical Sciences, [B]Mechanical Engineering, [1]University of Victoria, Victoria, BC, Canada

As they can both self renew and differentiate into any cell type, pluripotent stem cells provide an excellent starting point for tissue engineering. In addition to the more commonly used embryonic stem (ES) cells, induced pluripotent stem (iPS) cells provide an exciting alternative for tissue engineering applications. IPS cells are generated from adult somatic cells, such as skin cells, by inducing specific transcription factors that restore pluripotency. The use of iPS cells allows for the generation of patient-specific stem cell lines by converting a patient's differentiated cells back into a pluripotent state. Thus, it is theoretically possible to combine iPS cells with biomaterial scaffolds to engineer patient-specific tissues. Towards achieving this goal, the Willerth lab has been investigating the behavior of neural progenitors derived from both mouse and human ES and iPS cell lines inside of different types of biomaterial scaffolds in order to produce neural tissue that could replace damaged regions of spinal cord present after injury and restore lost functionality. This current study investigates the behavior of neural progenitors derived from pluripotent stem cells inside of 3D fibrin scaffolds. Fibrin is a naturally derived protein-based biomaterial commonly used for tissue engineering applications. For the ES cells, we used the mouse R1 ES cell line (Nagy Lab) and the human H9 cell line (WiCell). We obtained our mouse iPS cells from Systems Biosciences and the human iPS cell line 1-DL-01 from the Wisconsin International Stem Cell Bank. Our group researched methods of efficiently producing neurons from iPS cells when cultured inside of fibrin scaffolds. We investigated different methods of embryoid body formation with different chemical cues to determine their effects on stem cell behavior in these 3D microenvironments. To evaluate progress towards our goal of efficiently engineering reproducible neural tissue, we qualitatively assessed the differentiation state by staining for neuronal markers and further quantified the differentiation state

with flow cytometry.. Overall, this novel approach to neural tissue engineering could result in the development of a clinically relevant, patient-specific therapy for the treatment of spinal cord injury.

T-2291

POTENCY TESTING FOR TISSUE ENGINEERED AND REGENERATIVE MEDICINE PRODUCTS

Kelly Guthrie, Andrew Bruce, Namrata Sangha, Deepak Jain, **Joydeep Basu**
Tengion Inc, Winston-Salem, NC, United States

Introduction: Potency is a critical quality attribute of biological products, defined as: *the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result*. A potency assay should leverage product mechanism of action (MOA). Alternatively, the assay should focus on a therapeutically relevant biological activity. Development of a systematic battery of parallel functional assays that, taken together, can forecast the *in vivo* molecular and cellular MOA relevant to the product regenerative outcomes is recommended. Such an approach is especially important during preclinical development. Here, we report development of an illustrative matrix of potency assays leveraging multiple MOA for Tengion's Neo-Kidney Augment (NKA), a regenerative renal cell-based product candidate for augmenting kidney function for end stage renal disease.
1) **MOA**: Regeneration of tubular function in diseased kidney by formation of new kidney tubules.
Assay: *de novo* self-organization of cellular product component into organoids composed of a 3D system of tubules.
Methods & Results: A key functional characteristic of NKA is self-organization into tubules and tubular networks within 24 hours upon culture in 3D hydro-gels composed of collagen(I) and/or collagen(IV). We show that NKA-derived tubules express key functional markers and are quantifiably linked to other indices of product potency.
2) **MOA**: Paracrine signalling through secretion of regenerative growth factors and micro-RNAs.
Assay: PCR/FACs-based quantitation of *mir-21* and other salient micro-RNAs
Methods & Results: We have specifically identified *mir-21* and other related micro-RNA species expressed by NKA involved in NF-κβ and other key signaling pathways modulated by renal disease and regenerative bioactivity. We show that intra-renal delivery of NKA decreases NF-κβ nuclear localization within the rodent 5/6 nephrectomy model of kidney disease, an activity which may be mimicked *in vitro* with NKA-derived conditioned media. Presence of *mir-21* and related micro-RNA species within NKA as evaluated by PCR and Smart-flare identifies *mir-21* as a potential surrogate marker of NKA product potency.
3) **MOA**: Mobilization and migration of host-derived progenitor cells along the SDF1-CXCR4 axis.
Assay: Migration of NKA through a hydro-gel barrier in response to SDF1
Methods & Results: We demonstrate that migration of fluorescently labeled NKA cellular component within a defined time window through a hydro-gel barrier in response to recombinant SDF1 is an indicator of NKA potency. Additionally, we show that exposure to SDF1 is associated with up-regulation of CXCR4 in a quantifiable, dose-responsive manner.
4) **MOA**: *de novo* angiogenesis
Assay: Self-organization of HUVEC (endothelial cells) into 2D tubular structures in response to product-derived conditioned media
Methods & Results: Product may function in part by promoting the *de novo* assembly of vasculature through secretion of pro-angiogenic factors (VEGF). We demonstrate that assembly of tubular networks from HUVEC in 2D culture in the presence of NKA-derived conditioned media may be applied as a cell-based readout for functional VEGF secreted by NKA cellular component.
Conclusions: Taken together, this matrix of functional assays may facilitate evaluation of potency for Tengion's NKA cell-based therapeutic.

T-2292

DECELLULARIZATION AND CHARACTERIZATION OF HUMAN PERICARDIUM MEMBRANE AS A SCAFFOLD FOR CARDIAC TISSUE ENGINEERING

Sareh Rajabizeleti[1], Sasan Jalili Firoozinezhad[2], Fahimeh Khayyatan[2], Sadaf Vahdat[2], Mahnaz Azarnia[1], Hossein Baharvand[2], Nasser Aghdami[2]

[1]Department of Biology, kharazmi university (Tarbiat Moallem), Tehran, Iran, Islamic Republic of, [2]Department of Stem Cells and Developmental Biology, Royan Institute, Tehran, Iran, Islamic Republic of

Introduction:
Cardiovascular diseases hold the highest mortality rate among other diseases which shows limitations of common therapies. The 3D scaffolds have contributed mainly in treatment of heart failure postmyocardial infarction. Natural scaffolds which resemble more to the targeted damaged tissue, seems to be more appropriate for restoring damaged extracellular matrix and replacing the necrotic cardiomyocytes. In this study human pericardium membrane was studied as an appropriate scaffold for myocardial tissue engineering and compared to collagen type I scaffolds which applied in previous studies.
Materials and Methods:
Human pericardium Membrane (PM) was treated with standard decellularization solution consisting hypotonic lysis buffers. Tissues were rinsed in DPBS under gentle agitation at 4oC and then acellular PM (APM) was lyophilized up to 24 hours. In order to prepare the APM for cell seeding, thick fibrous Layer of, about 250 µm, was removed from it by cryostat. Rat tail Collagen (RC) gel was fabricated by freeze-drying method. In brief, 900 µL of solubilized RC gels were poured into each well were frozen at -80 ºC and lyophilized. The scaffolds were then chemically cross-linked for 24 h in 70% ethanol and 30mM EDC/NHS. Morphology of these scaffolds compared with Scanning Electron Microscopy (SEM) images. In order to compare the physico-mechanical properties of fabricated scaffolds, the mechanical and swelling properties of APM and RC were evaluated. To investigate the collagen and sulfated glycosaminoglycan (sGAG) content in these structures specific assays were applied. One-dimensional gel electrophoresis was performed on APM and compared to RC. Finally, in order to study the effect of these scaffolds on Sca-1+ cardiac progenitor cells; MTS assay, histological staining and SEM imaging were carried out for both samples after 3, 7 and 14 days in culture.
Results and Discussion:
Our results indicated that the number and size of porosities in APM significantly increased after lyophilisation. The swelling ratio of APM after 24 h was approximately 83% and 3 fold more than RC scaffolds. The Ultimate Tensile Strength (UTS) and Young's modulus of APM samples are significantly higher than RC scaffolds. The amount of GAG and Collagen is also much higher in APM scaffolds. The MTS assay showed that cells have a growth rate similar to control group on TCPS and RC in 3, 7 and 14 days after cell seeding. Histological and SEM microscopic results showed that cells attached on the surface of membrane and connected into ECM. With Masson's Trichrome (MT) staining collagen fibers were observed among cells and exhibited normal morphology.

T-2293

REPAIR OF KNEE LESION IN OVINE MODEL USING HUMAN DENTAL PULP STEM CELL ASSOCIATED WITH BIOMATERIAL.

Marcos VM Silva[1], Graciela Pignatari[1], Isabella Fernandes[1], Fabiele Russo[1], Rafael Agopian[1], Luiz Claudio Silva[1], Stefano Hagen[1], Maria Angélica Miglino[1], Daniel Seitz[2], Patricia Beltrão-Braga[1]

[1]Univ of São Paulo, São Paulo, Brazil, [2]Univ of Baireuth, Baireuth, Germany

Osteoarthritis (OA) is one of the diseases that affect more people worldwide leading to decreased quality of life. It mainly affects the knee joint and results in degradation of chondrocytes from the articular cartilage. In addition, the articular cartilage is avascular and presents a limited capability of regeneration. Consequently, there is no cure or effective treatment to Osteoarthitis.
In this study, we proposed a cellular therapy protocol using human dental pulp stem cells (HDPSC) to treat osteoarticular injury in ovine model. HDPSC are very well established, easy to obtain, exhibits good cellular plasticity and capacity of proliferation. In this project, the HDPSC were associated with a biomaterial made mainly with chitosan

and hydroxyapatite, specially manufactured for this study at Friedrich-Baur-Forschungs-Institute Für Biomaterilien, in Germany.
Four circular osteochondral defects (6x6 mm) were created in femorotibiopatelar's ovine joint and subsequently treated as follows: infusion of biomaterial alone, just the HDPSC, biomaterial associated with the HDPSC or no treatment (experimental control). After surgery, the animals were monitored clinically and under image analysis (X-ray, ultrasonography (US) and arthroscopy). Also, the animals recovered one week after surgery, returning to support the operated limb. The x-ray analyses showed that the biomaterial is properly inserted into the cleft created and do not cause any type of rejection reaction in the animals. The US and arthroscopy analyses revealed that HDPSC associated with the biomaterial produced an possible reconstitution of damaged tissue, confirmed by histology.
Based in our results, we suggest that HDPSC associated with this biomaterial could be a good way to treat osteoarticular injury.

Embryonic Stem Cell Pluripotency

T-2295

ADENOVIRUS EARLY REGION 1B-ASSOCIATED PROTEIN 5 **R**EGULATES SELF-RENEWAL AND PLURIPOTENCY OF HUMAN EMBRYONIC STEM CELLS IN THE NUCLEUS AND CELL SURFACE

Hong Seo Choi[1], Young Joo Jang[2], Hyun Min Lee[1], Won-Tae Kim[1], Cheorl-Ho Kim[3], Chun Jeih Ryu[1]
[1]Sejong unversity, Seoul, Korea, Republic of, [2]Dankook unversity, Cheonan, Korea, Republic of, [3]Sungkyunkwan unversity, Suwon, Korea, Republic of

Adenovirus early region 1B-associated protein 5 (E1B-AP5) is a multifunctional RNA-binding protein. In this study, we investigated the functional roles of E1B-AP5 in human embryonic stem cells (hESCs) by siRNA technology, chemical inhibitor and antibody treatments. E1B-AP5 knockdown impaired hESC self-renewal and pluripotency via defective
proliferation and preferential differentiation into α-fetoprotein-positive cells. E1B-AP5 knockdown blocked the cell cycle progression into S-phase through degradation of cyclin E and CDC25A. E1B-AP5 interacted with the ATR-interacting protein and single stranded DNA binding protein RPA32 in hESCs, and E1B-AP5 knockdown activated ATR/ChK1-dependent DNA
damage checkpoint signaling without any exogenous DNA damage agents, suggesting that E1B-AP5 may play a role in the firing of replication origin during the G1/S transition in hESCs. E1B-AP5 knockdown caused a marked decrease in the levels of activating phosphorylation of ERK and AKT and inhibitory phosphorylation of GSK-3β, indicating that E1B-AP5 regulates hESC self-renewal and pluripotency via the PI3K/AKT, Wnt/GSK-3β/β-catenin, and MAPK/ERK signaling pathways which have been known as major extrinsic signalings for hESC self-renewal and pluripotency. In the previous study, by using monoclonal antibody 57-C11, we found that E1B-AP5 was also expressed on the surface of hESCs. Chemical inhibitor studies showed that cell surface-expressed E1B-AP5 was under the control of the MAPK/ERK and Wnt/GSK-3β/β-catenin signaling pathways among above three signaling pathways. Treatment of 57-C11 further showed that cell surface-expressed E1B-AP5 regulates hESC self-renewal and pluripotency via the MAPK/ERK signaling pathway. These findings provide for the first time mechanistic insights into how E1B-AP5 regulates hESC self-renewal and pluripotency in the nucleus and cell surface.

T-2296

UNDERSTANDING EARLY FATE DECISIONS BY STUDYING HETEROGENEITY IN HUMAN PLURIPOTENT STEM CELLS

Sonam Bhatia, Carlos Pilquil, Jonathan Draper
Department of Biochemistry and Biomedical Sciences, McMaster Unviersity, Hamilton, ON, Canada

Human pluripotent stem cell populations are considered to be heterogeneous in nature, containing cells with varying differentiation potentials. Understanding this heterogeneity and the lineage commitment of various cell types

present within a pluripotent population would allow a complete exploitation of their clinical potential. We present here one of the few evidences of early fate determination in human embryonic stem cells (ESCs). Human ESC cultures exhibit a mosaic pattern of expression of the pluripotency marker REX1 (Reduced Expression 1, Zfp42). Rex1 expression could divide pluripotent mouse ESCs into two metastable, functionally distinct sub-populations. Using REX1-VENUS fluorescent reporter in human ESCs we have recently shown that unlike mESCs, human REX1 expression demarcates stable subpopulations with distinct molecular and functional properties. Loss of REX1 expression in hESCs represents one of the first fate decisions during differentiation of human ESCs, which is accompanied by a change in the epigenetic profiles of pluripotency and differentiation genes. Using co-immunoprecipitation of tagged human REX1 protein we show that it is associated with the polycomb repressive complex I (PRC1), a major epigenetic regulator. The data suggests that human REX1 acts in conjunction with the epigenetic machinery to, perhaps, regulate or maintain the epigenetic status of various development genes, resulting in varying cell fate commitments.

T-2297

THE ROLE OF MYC IN CONTROLLING INDUCED PLURIPOTENCY

Roberta Scognamiglio[1], Nina Cabezas[1], Larissa Carnevalli[2], Andreas Trumpp[1]
[1]Department of Stem Cells and Cancer, German Cancer Research Center, Heidelberg, Germany, [2]Heidelberg Institute for Stem Cell Technology and Experimental Medicine, HI-STEM, Heidelberg, Germany

Reprogramming of human and mouse fibroblasts to induced pluripotent stem (iPS) cells has been achieved by the overexpression of the four transcription factors Oct4, Sox2, Klf4, and c-Myc. The potential of iPS cells in medical and pharmaceutical applications is enormous and the generation of pluripotent stem cells directly from patients is the basis for many approaches towards regenerative medicine. The generation of human and mouse iPS cells with a three-factors cocktail that does not include Myc, is possible and even reduces the risk of tumorigenicity in iPS-derived chimeras (Nakagawa et al., 2007; Wernig et al., 2008). However, in the absence of ectopic Myc, the efficiency of reprogramming is dramatically reduced, demonstrating that ectopic and likely also endogenous Myc fulfils an important role in the reprogramming process.
Derivation and maintenance of mouse ES cells without serum, but in the presence of two inhibitors repressing the ERK and GSK3b pathways (2i + LIF), can stabilize ES cells in a more naïve, so called “ground state” of pluripotency. The ground state has been defined as a basal proliferative state that is free of epigenetic restriction and has minimal requirements for extrinsic stimuli (Nichols et al., 2009). Strikingly, we find that c-Myc protein expression in the naïve status is greatly reduced compared to cultures in serum + LIF. This suggests that Myc expression is induced during the earliest differentiation steps of “ground state” ES cells and may therefore inhibit the ground state. Moreover, this may suggest that Myc activity is not required for pluripotency, a hypothesis we are currently testing experimentally.

T-2298

THE TRUE IDENTITY OF NAIVE PLURIPOTENT STEM CELLS

Maaike Welling[1], Hsu-Hsin Chen[2], Javier Munoz[4], Lennart Kester[1], Jan Philipp Junker[1], Albert Heck[4], Lev Silberstein[3], Peter Kharchenko[5], Alexander van Oudenaarden[1], Niels Geijsen[1]
[1]Hubrecht Institute, Utrecht, Netherlands, [A]Center for regenerative medicine, [B]Center for Regenerative Medicine, [2]Massachusetts General Hospital, Boston, MA, United States, [3]Biomolecular Mass Spectrometry and Proteomics, Utrecht University, Utrecht, Netherlands, [4]Center for Biomedical Informatics, Harvard Medical school, Utrecht, MA, United States

Embryonic stem cells (ESCs) are pluripotent stem cells derived from the inner cell mass (ICM) of blastocyst embryos. ESCs have the capacity of indefinite self-renewal while retaining the ability to give rise to derivatives of the three embryonic germ layers. In mice, pluripotent stem cells are classified into two distinct states, “naïve” LIF-dependent

ESCs and “primed” bFGF-dependent epiblast stem cells (EpiSCs). The present gap in knowledge concerning the specific intermediate states leading to stable pluripotency *in vitro* may account for the unsuccessful attempts to stably maintain human pluripotent cells in a similar naïve state.
Although ESCs originate from the blastocyst ICM, the nature of these cells is still largely unknown. Experimental evidence suggests that ESCs stem from a subpopulation of emerging germ cells that develops within the explanted ICM and most ESC lines transiently express the early germ cell marker Blim1 during their derivation. Indeed, one of the hallmark characteristics of murine ESCs clearly distinguishing them from EpiSCs is the expression of a distinct germ cell signature, suggesting a close association with the early germline. Yet, a when ESCs are derived in the presence of a combination of two inhibitors, a GSK3β-inhibitor and a MEK-inhibitor (2i conditions), ESCs establishment does not proceed through the transient Blimp1 population suggesting there are two routes to ESC derivation. Using differential culture conditions, we have further explored the ontogeny of ESCs and their relationship to the germline.

T-2301

TRANSCRITIONAL LANDSCAPE OF TBX3 MEDIATED FUNCTIONAL INTERACTIONS IN THE MAINTANENCE OF THE MOUSE EMBRYONIC STEM CELL STATE

Avinash Waghray, Jun-Jun Ding, Ran Brosh, Julian Gingold, Betty Chang, Jianlong Wang, Ihor R. Lemischka
Development and Regenerative Biology, Mount Sinai Sch Of Medicine, New York, NY, United States

Tbx3, a T-box family protein, has been shown to be involved in the maintenance of the mouse embryonic stem (mES) cell self-renewal and pluripotency. It has also been shown that Tbx3 over-expression improves both the quality and efficiency of reprogramming using iPS and cell-fusion based reprogramming experiments. RNAi mediated knock down of Tbx3 in mES cells showed that Tbx3 may control the mRNA expression of a certain set of genes that do not change in their expression upon knock of Oct4, Sox2 or Nanog (OSN). We also found that OSN cluster binds to a different set of genes than Tbx3 alone. Other studies that extensively looked at the protein-protein interaction partners of Oct4 and Nanog did not find Tbx3 as their interaction partner. Given the evidence, we hypothesized that Tbx3 maintains the mES cell state through pathways distinct from those controlled by the OSN cluster.
To study the role of Tbx3 within the mES cell transcriptional network we used an affinity pull down strategy to identify the protein-protein interaction partners and genome-wide binding targets of Tbx3 in mES cells using IP-LC/MS-MS and ChIP-seq methodology. We found that Tbx3 binds to the genomic region of ES cells genes such as Oct4, Nanog, Sox2, Esrrb, Dppa3 and Sall4. It is also bound to the genomic region of genes involved in mesoderm (*Myo1b, T*) and ectoderm (*Neurog1, Neurog3*) development. Upon further analysis we found that Tbx3 binds to a large number of promoter or enhancer regions uniquely and some in combination with OSN cluster. Checking the gene expression analysis of the clusters indicated that the high mRNA expression of genes regulated at their enhancer and promoter regions was dependent significantly upon the binding of Tbx3 alone OR Tbx3 in combination with the OSN cluster.
We also identified ~100 proteins that seem to interact with Tbx3 in mES cells. Only a small subset of these proteins is found to interact with Oct4, Nanog and Esrrb. Tbx3 protein interaction partners are found to play roles in transcription, chromatin binding and mRNA processing pathways. A number of the proteins are regulated at either their promoters or enhancers by key mES cell genes such as Nanog, Oct4, Sox2, Esrrb, Tcfcp2l1 and Tbx3. We also found that a significant number of these proteins effect the self-renewal state of mES cells in genome-wide RNAi studies. We knock down a number of the Tbx3 protein-protein interaction partners using shRNAs and found that they maintain the ES cell state. We therefore constructed a transcriptional landscape controlled by Tbx3 in mES cells and integrated it into the existing mES cell network. We hope to further indentify functional modules within this genome-wide network that elucidate the function of Tbx3 in the maintenance of mES self-renewal and pluripotency.

T-2302

DECELLULARIZED MATRIX FROM FIBRONECTIN ENGINEERED FEEDER CELLS FOR HUMAN EMBRYONIC STEM CELLS EXPANSION

Daekee Kwon[1], Gwang-Sik Kang[1], Inbo Han[1], Kwideok Park[2], Soo-Hong Lee[1]
[1]CHA University, Gyeonggi-do, Korea, Republic of, [2]Korea Institute of Science and Technology, Seoul, Korea, Republic of

Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) are a hot issue in the field of cell replacement therapy. Feeder-free culture condition would be prerequisite for expansion of clinical grade hESCs and hiPSCs. However, current feeder-free culture condition has a limitation on cost-effective and large scale expansion. The aim of this study is to develop novel feeder-free culture system for hESCs expansion by decellularizing extracellular matrix (ECM) engineered feeder cells. Human fibronectin (hFN; hFibronectin-12.3kb), human collagen I (hCOLI; hCOLIAI-10.3kb and hCOLIAII-10.0kb) and human collage II (hCOLII; hCOLIIAI-10.4kb) gene expression vectors were constructed using pTracer-EF/V5-HisA vector. These vectors were transfected to 293T cells by PEI and then the overexpression of hFN, hCOLI and hCOLII was confirmed by qRT-PCR. The fold increase of transcripts was found to be 36.89±8.24 (hFN), 100.66±30.67 (hCOLI) and 85.14±6.36 (hCOLII) than control. According to FACS analysis, the ratio of transgene expressing cells is 88.97% (hFN), 97.38% (hCOLI) and 88.22% (hCOLII) respectively. When the H9 hESCs were cultured on the decellularized hFN, hCOLI and hCOLII engineered matrix for 12 days with mTeSR medium, Oct-4 expression was highest at hFN matrix compared to others (hCOL, hCOLII and control). It concludes that decellularized hFN engineered matrix showed the significantly higher potency to maintain pluripotency of hESCs than hCOLI, hCOLII and control. Various engineered matrix could be valuable source for large-scale production of clinical grade hESCs. In addition, it could provide a valuable model system for the study of interaction between ECM and hESCs.

T-2303

SUPER-ENHANCERS AT KEY CELL IDENTITY GENES

Denes Hnisz, Warren A. Whyte, David A. Orlando, Brian J. Abraham, Charles Y. Lin, Peter B. Rahl, Tong Ihn Lee, Richard A. Young
Whitehead Institute for Biomedical Research, Cambridge, MA, United States

Much of the transcriptional control of mammalian development is due to the diverse activity of transcription factor-bound enhancers that control cell-type specific patterns of gene expression. In embryonic stem cells (ESC), the master transcription factors Oct4, Sox2 and Nanog bind enhancer elements and recruit the Mediator co-activator to activate much of the gene expression program underlying the pluripotent state. We report here that the ESC master transcription factors and Mediator form “super-enhancers” at most genes known to control the pluripotent state, including those encoding the master transcription factors themselves. These super-enhancers consist of extraordinarily large genomic domains occupied by exceptional amounts of Oct4, Sox2, Nanog, Klf4, Esrrb and Mediator. Super-enhancers stimulate considerably higher transcription than typical enhancers in vivo and in reporter vectors. Reduced levels of Oct4 or Mediator cause preferential loss of expression of super-enhancer-associated genes relative to other genes, suggesting how changes in gene expression programs might be accomplished during development. We also found super-enhancers containing cell type-specific master transcription factors at genes that define cell identity in more differentiated cells. These results implicate super-enhancers in the control of mammalian cell identity and differentiation.

T-2305

RIF1 REGULATES 2-CELL EMBRYO STATE AND TELOMERE HOMEOSTASIS IN MOUSE EMBRYONIC STEM CELLS

Lin Liu[1], Jiameng Dan[2], Na Liu[2], Yifei Liu[3], Maria Chiourea[4], David L. Keefe[5], Lingyi Chen[2], Sarantis Gagos[4], Andrew Xiao[3]
[A]Department of Cell Biology and Genetics, [B]Cell Biology and Genetics, [1]Nankai University, Tianjin, China, [2]Yale Stem Cell Center and Department of Genetics, Yale University School of Medicine, New Haven, CT, United States, [3]Biomed-

ical Research Foundation of the Academy of Athens Greece (BRFAA), Athens, Greece, [4]New York University Langone Medical Center, New York, NY, United States

Telomere-associated protein Rif1 was originally identified in yeast as a RAP1 interacting factor that negatively regulates telomere length, and plays an important role in regulation of telomerase recruitment to yeast telomeres. However, mammalian *Rif1* has been found no known function at telomeres but appears to be involved in DNA damage responses and replication timing. *Rif1* deficiency *in vivo* leads to embryonic lethality, indicating that *Rif1* is critical for early embryo development. Notably, *Rif1* is highly expressed in mouse embryonic stem (ES) and germ cells, and also is a target of Oct4, Sox2 and Nanog, implicated in ES self-renewal; however, its function in stemness still remains elusive. Interestingly, ES cell pluripotency fluctuates with 2-cell (2C) embryo state, yet the regulatory mechanisms and functional consequences of the sporadic activation of 2C-genes are obscure. We show that *Rif1* is required to regulate the sporadic activation of 2C-state in ES cell cultures. *Rif1* mediates heterochromatic histone silencing to suppress over-activation of 2C-state in ES cells. Furthermore, *Rif1* represses excessive expression of *Zscan4*, an important 2C-gene, to maintain telomere length homeostasis and genomic stability. Excessive expression of *Zscan4* following *Rif1* depletion leads to telomere hyper-recombination and chromosomal instability, in association with reduced levels of Oct4. Restricted sporadic activation of *Zscan4 in 3-5% of ES cells represents a major* functional significance of 2C-state fluctuations in ES cell cultures. We suggest that *Rif1* plays novel roles in epigenetic regulation of 2C-state and maintenance of telomere homeostasis for ES cell self-renewal and pluripotency.

T-2307

IN SILICO PREDICTION AND VALIDATION OF PLURIPOTENT STATE TRANSITIONS AND SINGLE CELL HETEROGENEITY

Ayako Yachie, Kento Onishi, Peter W. Zandstra
Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada

There are different types of pluripotent stem cells such as embryonic stem cells (ESC) and epiblast stem cells (EpiSC), which have distinct characteristic gene expression patterns and require distinct signaling inputs to sustain pluripotency. Transcription factors (TFs) responsible for stabilizing pluripotency, including Nanog, Oct4 and Klf4, are known to undergo temporal fluctuations at the single-cell level. Furthermore, we and others have shown that the forward and reverse transition between pluripotent cell state, including ESC and EpiSC, can be encouraged by manipulations the cell microenvironment and specific signaling pathways including LIF and FGF. Despite significant effort, the molecular basis and consequence of TF fluctuation and the role of this molecular heterogeneity in the transitions between pluripotent cell states (and indeed in differentiation propensities), remains unclear.
To address this we have developed a novel simulation approach to predict population-level pluripotent cell state changes by mimicking the time-dependent single cell state transitions based on an underlying gene regulatory network (GRN) predicted from the data-mining of over 1,000 published microarray expression data of mESC.
Using our consensus GRN structure, a Boolean model comprising 25 core TFs as well as seven signaling pathways and 139 regulatory interactions in mESC was developed. Importantly, our GRN structure is designed to evolve as a function of input from key signaling molecules, including LIF, BMP4, Activin A and FGF and Wnt- /ERK- signal modulating factors. A random asynchronous update approach has been taken for simulating the Boolean model network states. By starting from the various initial boolean states, the probabilities of the state transitions is obtained through an iterative process. To capture the observation that TF expression patterns are variable and reversible at the single-cell level, we defined the strongly connected components in the Boolean state-transition graph as unique solutions representing maximum set of individual pluripotent cells where any single cell state is reachable from any other state under the above regulatory rules.
To validate the model and its predictions, population-level gene expression patterns from several combinations of signaling inputs were predicted and compared with those obtained from experimental data. The simulation successfully predicted the distinct gene expression pattern between ESC and EpiSC shown in RNA-seq data and microarray gene expression data, only by changing the signaling inputs to the model (from LIF to Activin A + FGF).

Current efforts are focused on the evaluation of predicted features including removing feedback inputs into Nanog, and screening the effects of different signaling inputs (growth factors) on predicted stable pluripotent cell states and their transitions.
In summary, using the novel simulation methodology and the GRN model developed in this study, we can predict the effects of input signal combinations on pluripotent cell fate transitions and gain novel insights into the role of TF fluctuations on single cell and population level cell fate control.

T-2308

AN RNAI SCREEN IDENTIFIES NOVEL PROTEIN KINASES AND PHOSPHOTASES REQUIRED FOR PLURIPOTENCY OF HUMAN EMBRYONIC STEM CELLS

Hongwei Zhou, Francesco Faiola, Arven Saunder, Jianlong Wang, Ihor Lemischka
Icahn School of Medicine at Mount Sinai, New York, NY, United States

One of the most important issues in stem cell field is
the mechanism that regulates self-renewal and pluripotency, two crucial aspects
of stem cell function. Although considerable information defining the molecular
and biochemical regulatory networks responsible for initiating and maintaining
the pluripotent “state” are available in mouse embryonic stem cells (mESCs),
such information is much more sparse for the human (h) ESCs. It has been shown
that activation of the canonical Wnt pathway is sufficient to maintain
pluripotency of both mESCs and hESCs, however, the signaling pathways that
maintain hESCs in an undifferentiated state are not fully explored. Protein
kinases (PKs)- and protein phosphatases (PPases) play central roles in
signaling transduction. Our objective is to identify novel human PKs and PPases
essential for maintenance of hESC pluripotency. We performed a “loss-of-function”
shRNAs library screen with 1839 lentiviral shRNAs targeting ~700 PKs and PPases
genes in hESCs. We employed FACS sorting
approach to select targets. Gene
Frequency Analysis was applied to classify top candidate genes. Top 25
PKs and PPases candidate genes from our primary screen were further validated
in the secondary screen. Three top hits were confirmed to play critical roles
in hESC pluripotency from both phenotypic analysis and functional validation.
One of the top hits, CSNK1A1 was chosen for further study. Our results
suggested that CSNK1A1 gene knockdown not only compromised hESC maintenance but
also impeded human fibroblasts reprogramming. We are investigating the
mechanisms by which CSAK1A1 gene regulates hESCs self-renewal and pluripotency,
and attempt to elucidate novel signaling pathways that control stem cell
pluripotency and somatic cell reprogramming in the human system.

T-2311

THE H3K9 DEMETHYLASE JMJD1C SUSTAINS MIR-302 EXPRESSION AND REPRESSES NEURAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

Ren-He Xu, Jianle Wang, Xiaofang Wang, Jung W. Park
Genetics and Developmental Biology, Univ of Connecticut Hlth Ctr, Farmington, CT, United States

It has been recently reported that the pluripotency factor OCT4, the early neural inducing factor NR2F2, and the pluripotency-associated miRNA miR-302 are linked in a regulatory circuitry that critically regulate both pluripotency and neural differentiation of human embryonic stem cells (hESC). We show here that JMJD1C, a H3K9 demethylase expressed in undifferentiated hESC, plays a key role in the regulatory circuitry. hESC with JMJD1C knockdown (KD)

retain the state of self-renewal and pluripotency, but express lower miR-302 than control hESC. JMJD1C directly binds to the miR-302 promoter in hESC. Upon withdrawal of bFGF (an inhibitor of neural initiation) from a defined culture medium, the KD, but not control, hESC differentiate into neural progenitors within three days - the fastest ever reported, accompanied by rapid increase of NR2F2 expression. A miR-302 analogue or an inhibitor of H3K9 methylation reduces neural induction from the KD hESC, whereas a miR-302 inhibitor promotes hESC differentiation. Together, our findings suggest that JMJD1C plays a central role in control of neural differentiation from hESC, which involves histone demethylation of miR-302 to sustain its expression, and that inhibition of JMJD1C is sufficient to rapidly induce neural progenitors from hESC in the defined medium depleted of bFGF. This is also the first evidence, to our knowledge, for epigenetic modification of miR-302 in hESC.

T-2312

DISSECTING THE ROLE OF LIF/STAT3 SIGNALING IN THE MAINTENANCE AND INDUCTION OF PLURIPOTENCY.

Graziano Martello, Austin G. Smith
WT-MRC Stem Cell Institute, Cambridge University, cambridge, United Kingdom

Embryonic stem (ES) cells and induced-pluripotent stem (iPS) cells can differentiate, in vitro and in vivo, into all the cell types of the adult organism, an ability defined as Pluripotency. LIF and its downstream effectors Stat3 have been identified as key players in the maintenance, and induction, of Pluripotency, but the molecular mechanism still remains only partially understood.
The aim of my project is the identification of direct targets of Stat3 and the characterization of those functionally relevant for pluripotency.
We used Chromatin Immunoprecipitation followed by deep sequencing (CHIP-seq) to identify genes directly bound by Stat3. We then performed transcriptome analysis to find genes differentially expressed upon LIF stimulation.
By intersecting the data from the 2 approaches we identified direct Stat3 targets (i.e. genes directly bound and regulated by Stat3). Among the direct targets we then selected candidates for functional validation.
We identified 3 transcription factors, Klf4, Gbx2 and Tcfcp2l1, as key mediators of pluripotency downstream of LIF/Stat3 signal. Over-expression of these factors is sufficient to maintain Pluripotency in the absence of LIF in ES cells, with Tcfcp2l1 showing a stronger biological activity. Knockdown of Tcfcp2l1 resulted in impaired self-renewal; intriguingly, Klf4 and Gbx2 downregulation could be tolerated by ES cells. Moreover, we found that Gbx2 and Tcfcp2l1 are upregulated during the early phases of reprogramming and that Tcfcp2l1 downregulation caused a dramatic reduction in reprogramming efficiency.
Our study identified Tcfcp2l1, Klf4 and Gbx2 as key mediators of LIF/Stat3 signalling in Pluripotent cells.

T-2313

LIN28 REGULATES MITOCHONDRIAL METABOLISM IN EMBRYONIC STEM CELLS AND CANCER CELLS

Jin Zhang, Shyh-Chang Ng, Sutheera Ratanasirintrawoot, Marc Seligson, Zhaoting Wu, Ho-chou Tu, Hao Zhu, George Q. Daley
Children's Hospital Boston / Havard Medical School, Boston, MA, United States

Lin28 is a RNA binding protein that is highly expressed in early embryogenesis, silent in most adult tissues, and re-activated in multiple human cancers. Transgenic mice that over express Lin28 have increased body size, delayed puberty and increased glucose tolerance, while Lin28 knockout mice are runted and show insulin resistance. Lin28 binds and inhibits biogenesis of let-7 family microRNAs, which repress multiple genes in the insulin and mTOR signaling pathway. The mechanism by which Lin28 regulates glucose metabolism and insulin resistance is partially through inhibition on let-7, but we and others have shown that Lin28 can also bind a variety of mRNAs to selectively enhance or repress their translation. In embryonic stem (ES) cells, we have found that the mRNAs of many metabolic genes involved in mitochondrial oxidative phosphorylation (OXPHOS) are direct targets of Lin28 binding. Knocking down Lin28 in wild type ES cells leads to deregulation of mitochondrial oxygen consumption, a preference for energy production from OXPHOS, and metabolic flux for biosynthesis. Similar changes were observed in

Lin28-deficient DGCR8-/- ES cells which do not produce mature let-7, suggesting that Lin28 can exert its metabolic effects independently of let-7. In addition, in cancer cells with Lin28 reactivation, such as neuroblastoma and lung cancer cell lines, knocking down Lin28 leads to lower mitochondrial function; while in cancer cells not expressing Lin28, such as Hela cells, ectopic over expression of Lin28 leads to enhanced OXPHOS function. These findings implicate Lin28 as a major metabolic regulator in pluripotent stem cells and cancer. Altered cancer metabolism by Lin28 might provide new therapeutic targets for drug screening.

T-2314

LOW-GRADE MOSAICISM IN HUMAN EMBRYONIC STEM CELL CULTURES

Kurt Jacobs, Afroditi Mertzanidou, Mieke Geens, Karen Sermon, Claudia Spits
Vrije Universiteit Brussel, Brussels, Belgium

It is well known that human embryonic stem cell (hESC) cultures frequently become chromosomally abnormal, most of the abnormalities being recurrent. Previous studies report high frequencies of low-grade mosaicism in hESC cultures. Strikingly, depending on the technique used, aneuploidy frequencies (predominantly chromosome loss) and other genomic abnormalities vary strongly, ranging from zero to 35%. In this work, we studied the genetic composition of 25 cells of VUB02 at passage 59 and 29 at passage 351 by single-cell array-based comparative genomic hybridization. This technique has a higher resolution than conventional cytogenetic methods, allowing the detection of small regions of gains and losses at the single-cell level, providing insight into the levels of low-grade mosaicism of these cultures. We found that 44% of the cells contained at least one genomic region with abnormal copy number. The vast majority of the abnormalities comprised gains and losses of small chromosome segments (between 0.27 and 32.12 Mb), most of them unique to one cell. Also, one full chromosome 20 gain was detected. These results were confirmed on 1337 cells using interphase FISH for chromosome 18 with a centromeric and two telomeric probes. Our results suggest that hESC frequently undergo DNA damage that is incorrectly or not repaired, but that these aberrations are mostly rapidly cleared from the culture.

T-2315

MICRORNAS TARGET CHROMATIN MODIFIERS AND CELL CYCLE GENES TO MAINTAIN SELF-RENEWAL IN MOUSE EMBRYONIC STEM CELLS

Betty Chang[1], Marek Kudla[2], Gregory J. Hannon[2], Ihor R. Lemischka[1]
[1]Black Family Stem Cell Institute, Mount Sinai School of Medicine, New York, NY, United States, [2]Watson School of Biological Sciences, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, United States

MicroRNAs (miR) bind in a sequence specific manner to mRNAs, often within the 3'UTR where pairing is mediated by the RISC complex (of which Ago2 is a key member) where this interaction subsequently disrupts transcript translation. The collapse of the miR biogenesis pathway by occlusion of DGCR8 or Dicer in mouse embryonic stem cells (mESC) alters the cell cycle and impairs differentiation potential. To gain further insight into microRNA activity within self-renewal and pluripotency we examine global changes in miR and gene expression by deep sequencing of mESCs during single transcription factor, Nanog perturbation. Previous ChIP studies have identified Nanog as binding to several miR gene putative transcript start sites, including the TSS of the 290 and 302 cluster families. We further perform Ago2 immunoprecipitation followed by high through put sequencing (HITS-CLIP) to characterize the direct miR-mRNA target relationship.
We confirm previous reports of 290/302 cluster miR expression in mESCs, where they dominate >50% of the total miR reads identified. These miRs are characterized as ESC specific in expression and are reported to target cell cycle genes to maintain self-renewal and promote induced pluripotent stem cell (iPSC) reprogramming when introduced into fibroblasts. Upon differentiation triggered by Nanog knockdown, the 290 cluster miRs decline in the total miR population from ~60 to ~25%, however, the same family members continue to comprise ~75% of all miRs identified as Ago2 associated, falling only to 65% at the end of the time course. Although these miRs appear to be transcrip-

tionally down regulated, miR turn over or degradation does not occur at the same rate, and remain incorporated in the RISC complex.
Our Ago2-HITS-CLIP of mESCs reveals a variety of mRNA transcript binding, here we focus on the 3'UTRs, many of which harbor TargetScan predicted sites. We find a number of genes with known epigenetic or chromatin modifying function, e.g. Suz12 of the PRC2 complex and known cell cycle regulators, e.g. p21. Within the 3'UTRs, we find enrichment of reads corresponding to the predicted TargetScan miR binding sites of mmu-miR-19 for Suz12 and the seed corresponding to many members of the 290/302 miRs for p21.
To confirm the role of miRs on the HITS-CLIP identified chromatin modifier and cell cycle related gene sets, we examine miR ablated Dicer-null mESCs. These cells exhibit slower proliferation rates in comparison to wild-type mESCs and are unable to differentiate upon LIF deprivation. The expression profile of Suz12 and p21 is elevated in the Dicer-null mESCs with reference to wild-type mESCs, indicative of a release of miR dampening. We use miR mimics introduced into these Dicer-null mESCs to affirm alteration to gene expression.
To augment our understanding of these genes in self-renewal and pluripotency, we monitor expression via miRseq and mRNAseq during reprogramming of mouse embryonic fibroblasts (MEF) by a Tet-inducible Pou5f1, Sox2, Klf4 and c-Myc cassette. We observe a coordinated up regulation of pluripotency-related 290/302 miRs during iPS formation. Several of the chromatin modifier and cell cycle related genes discovered by HITS-CLIP also exhibit differential expression with decreased levels at later stages in reprogramming.
We propose microRNAs play a vital role in the maintenance of self-renewal and pluripotency by regulating chromatin modifiers and cell cycle genes to fine-tune the cellular landscape in response to cell fate switches.

T-2316

EXPRESSION PROFILING AND IDENTIFICATION OF KNOWN AND NOVEL MIRNAS IN HUMAN EMBRYONIC STEM CELLS BY HIGH-THROUGHPUT SEQUENCING

Suvi Asikainen[1], Liisa Heikkinen[2], Juuso Juhila[3], Frida Holm[1], Jere Weltner[3], Ras Trokovic[3], Milla Mikkola[3], Sanna Toivonen[3], Diego Balboa Alonso[3], Iiris Hovatta[3], Garry Wong[2], Timo Otonkoski[3], Outi Hovatta[1]
[1]Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden, [2]A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland, [3]Research Program of Molecular Neurology, University of Helsinki, Helsinki, Finland

MicroRNAs (miRNAs) play a central role in stem cell biology as the in vitro delivery of a handful of stem cell-specific miRNAs is able to induce pluripotency in human and mouse somatic cells. In order to better understand the characteristics of embryonic stem cell miRNAs, we performed Illumina's small RNA-seq to profile and identificate known and novel miRNAs emerging from miRNA precursor (pre-miRNA)-containing hairpin loci in two human embryonic stem cell (hESC) lines (HS401 and HS181) and one newborn human foreskin fibroblast (hFF) line HFF-1. Three small RNA-seq libraries were prepared, sequenced and filtered to remove poor quality reads. 36-43 % of sequences in the hESC samples and 71% in the hFF sample map to known human miRNA hairpin loci. Differential expression analysis of in-house made Java script tool-extracted mature miRNA sequences revealed that the stem cell-specific miR-302-367 cluster, which is known to form the major hESC miRNA family, indeed forms a large portion of miRNAs in the hESC samples. Let-7 cluster predominates in the hFF. In addition, 21 candidate novel miRNAs which represent a predicted minor form for previously known miRNAs and 23 novel hairpins containing novel sequence in one arm (18 hairpins) or both arms (5 hairpins) were detected. Expression of three hESC-specific and two hFF-selective miRNAs were verified by quantitative real-time PCR and functional experiments for a selection of candidate novel miRNAs are ongoing.

T-2317

IDENTIFICATION AND CHARACTERIZATION OF MIR-135B AND MIR-363 AS NOVEL PLURIPOTENT CELL-SPECIFIC MIRNAS.

Lukasz Smorag, Wolfgang Engel, D.V. Krishna Pantakani

Human Genetics, University of Goettingen, Goettingen, Germany

MicroRNAs (miRNAs) are a novel class of small non-coding RNAs which function in several cellular processes through either the inhibition of target mRNA translation or even the degradation of the transcript. Recent studies have clearly shown that miRNAs are involved in both establishment and maintenance of pluripotency as well as in their differentiation potential into specific cell lineages. In the present study, we performed a comparative miRNA expression analysis (miRNAome) between undifferentiated and differentiated cells and identified miRNA-135b and miRNA-363 as novel candidates of pluripotency regulation. Subsequent qRT-PCR analysis on several pluripotent cell types confirmed that these two miRNAs are expressed specifically in all pluripotent cell types but not in somatic cells/tissues. The DNA methylation analysis and chromatin immunoprecipitation studies revealed that the promoter regions of miR-135b and miR-363 are epigenetically active in pluripotent cells. The expression analysis during somatic cell reprogramming revealed that these two novel miRNAs are expressed along with the known pluripotent cell-specific miR-290 members at day 7 of reprogramming, further suggesting their role in establishment and maintenance of pluripotency. Moreover, we performed *in-silico* analysis to identify the respective mRNA targets of these miRNAs and the results highlight that miRNA-135b targets genes involved in the cell cycle regulation, whereas miRNA-363 targets are implicated in differentiation processes. Further functional studies revealed that miR-135b modulates the Wnt-signaling pathway to maintain the pluripotency, whereas miR-363 was found to block the early cardiac differentiation pathway of pluripotent cells. Interestingly, elevated expression of miR-135b was described previously in several cancers, suggesting the existence of common molecular mechanisms regulating the cell cycle between pluripotent and cancer cells.

T-2318

ENHANCER COMMISSIONING BY H3K4 METHYLTRANSFERASE MLL4 REGULATES ES CELL PLURIPOTENCY

Chaochen Wang[1], Ji-Eun Lee[1], Shiliyang Xu[1], Young-Wook Cho[2], Todd Macfarlan[3], Kairong Cui[4], Chengyu Liu[4], Kai Ge[1]

[1]LERB, NIH/NIDDK, Bethesda, MD, United States, [2]Chuncheon Center, Korea Basic Science Institute, Chuncheon, Korea, Republic of, [3]NIH/NICHD, Bethesda, MD, United States, [4]NIH/NHLBI, Bethesda, MD, United States

In eukaryotes, gene regulation involves cooperation between transcription factors and chromatin modifiers on genomic elements including enhancers. Enhancers are marked by mono- and di-methylation of histone H3 lysine 4 (H3K4me1/2). Active enhancers are further marked by acetylation on histone H3 lysine 27 (H3K27ac). However, the methyltransferases responsible for H3K4me1/2 on mammalian enhancers have remained elusive. Further, the roles of H3K4 mono- and di-methyltransferases in enhancer activation and gene expression in embryonic stem cells (ESCs) are unknown. Here we show that MLL4 is a major H3K4 mono- and di-methyltransferase in mouse ESCs. ChIP-Seq analysis in ESCs reveals that MLL4 is enriched on enhancers and co-localizes with pluripotency circuitry transcription factors Sox2 and Oct4 on enhancers. MLL4 physically interacts with Oct4 in ESCs. Deletion of *MLL4* in ESCs eliminates H3K4me1/2 and attenuates H3K27ac on MLL4-positive enhancers, indicating that MLL4 is required for enhancer activation. Further analysis of direct MLL4 target genes identifies multiple Oct4-regulated genes important for ESC pluripotency. Deletion of *MLL4* impairs activation of Oct4-binding enhancers on these genes and leads to decreased gene expression. Consistently, MLL4 is required not only for maintaining ESC identity but also for reprogramming of somatic cells into induced pluripotent cells (iPSCs). Finally, *MLL4* knockout ESCs also show severe defects in differentiation towards mesoderm and endoderm, indicating that MLL4 is required for ESC pluripotency. Our results thus identify an essential role of H3K4 mono- and di-methyltransferase MLL4 in enhancer commissioning on genes critical for establishing and maintaining ESC pluripotency.

T-2321

DISSOCIATION OF THE INTEGRIN β4 AND PLECTIN COMPLEX BY GLUCOSAMINE CONTRIBUTES TO MOUSE EMBRYONIC STEM CELL MIGRATION AND PROLIFERATION: INVOLVEMENT OF SP1/CAM AND AKT/GSK-3β/SNAIL1

Ji Hoon Jeon, Han Na Suh, Mi Ok Kim, Seung Pil Yun, Jung Min Ryu, Bit Na Seo, Ho Jae Han
College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul, Korea, Republic of

Dissociation of the integrin β4 and plectin complex by glucosamine contributes to mouse embryonic stem cell migration and proliferation: Involvement of Sp1/CaM and Akt/GSK-3β/Snail1

Ji Hoon Jeon, Han Na Suh, Mi Ok Kim, Seung Pil Yun, Jung Min Ryu, Bit Na Seo, Ho Jae Han *
Department of Veterinary Physiology, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul 151-741, Republic of Korea
***Corresponding author:** Ho Jae Han D.V.M., Ph.D.
Department of Veterinary Physiology, College of Veterinary Medicine, Seoul National University, Seoul, 151-741, South Korea
Tel: 82-2-880-1261
Fax: 82-2-880-2732
E-mail: hjhan@snu.ac.kr

Abstract
We investigated the role of glucosamine (GlcN) on the integrin (IN) β4/plectin complex and its role in the regulation of mouse embryonic stem cell (mESC) migration and proliferation. GlcN decreased IN β4 mRNA/protein expression and IN β4/plectin complex association, which increased cell migration. GlcN increased intracellular calcium influx and protein kinase C (PKC) phosphorylation followed by IN β4 serine phosphorylation and dissociation of the IN β4/plectin complex. GlcN also increased O-GlcNAc transferase-dependent IN β4/plectin complex dissociation. Moreover, GlcN increased glycosylation and phosphorylation of specificity protein 1 (Sp1), and nuclear translocated Sp1 stimulated calmodulin (CaM) expression, which combined with plectin. In addition, GlcN increased Akt glycosylation and glycogen synthase kinase-3β (GSK-3β) phosphorylation as well as Snail1 glycosylation. Snail siRNA reversed the decrease in the IN β4/plectin complex and cell junctions (tight and adherent junction). GlcN increased cell migration, cell cycle regulatory proteins (CDK2/4, CyclinE/D1), and the percentage of S phase cells, which were inhibited by a PKC inhibitor, CaM1/2, or Snail1 siRNA. Additionally, GlcN maintained the undifferentiation markers of ESCs and did not affect differentiation markers. In conclusion, GlcN contributed to migration and proliferation of mESCs through dissociation of the IN β4/plectin complex via Ca^{2+}/PKC as well as the Sp1/CaM and Akt/GSK-3β/Snail1 signaling pathway.

T-2322

IDENTIFICATION OF A NOVEL COMPONENT REGULATING ES CELL SELF-RENEWAL AND PRE-IMPLANTATION DEVELOPMENT

xiaofeng zheng, Brad Lackford, Yiliang Miao, Williams Carmen, Guang Hu
NIEHS, RTP, NC, United States

Embryonic stem cells (ESCs) have two unique characteristics: self-renewal and pluripotency. To systematically study the molecular basis of self-renewal and pluripotency, we have previously carried out a genome-wide RNAi screen in mouse ESCs. We identified over 100 genes whose down-regulation caused differentiation, including genes encoding members of the Ccr4-Not protein complex. The Ccr4-Not complex is known for the regulation of transcription and mRNA stability, and has been implicated in various cellular activities such as DNA repair, spindle positioning, microtubule length regulation, and histone methylation.
Here we show that three components of the Ccr4-Not complex, Cnot1, Cnot2, and Cnot3, are important for maintaining mouse ESC identity. Genes corresponding to these three proteins are highly expressed in ESCs and down regulated during differentiation, and they are also enriched in the inner cell mass of the blastocyst stage embryos. In mouse ESCs, global gene expression analysis indicated that silencing Cnot1, Cnot2, or Cnot3 induces differentiation predominantly into the trophectoderm lineage. Interestingly, the Cnot genes do not impinge on other known self-renewal transcription factors or pathways. Instead, they maintain mouse ESC self-renewal by repressing the expression of early trophectoderm transcription factors such as Cdx2. Structure function analysis showed that the

C-terminal domains in the Cnot genes are important for their interactions and function in maintaining ESCs. Consistent with the results in ESCs, we found that Cnot3 is required for mouse pre-implantation development and its silencing impairs blastocyst formation ex vivo. Together, our data indicate that Cnot1, Cnot2, and Cnot3 represent a novel component of the core self-renewal and pluripotency circuitry in ESCs, and our approach illustrates the power of RNAi and forward genetics for the systematic study of ESC biology.

T-2323

DELPHINIDIN PREVENTS HYPOXIA-INDUCED MOUSE EMBRYONIC STEM CELL APOPTOSIS THROUGH REDUCTION OF INTRACELLULAR REACTIVE OXYGEN SPECIES-MEDIATED ACTIVATION OF JNK AND NF-κ B, AND AKT INHIBITION

Bit Na Seo, Mi Ok Kim, Han Na Suh, Jung Min Ryu, Seung Pil Yun, Ji Hoon Jeon, Ji Young Oh, Min Sik Yong, Jin Yi Han, Ho Jae Han
Department of Veterinary Physiology, Seoul National University, Seoul, Korea, Republic of

DELPHINIDIN PREVENTS HYPOXIA-INDUCED MOUSE EMBRYONIC STEM CELL APOPTOSIS THROUGH REDUCTION OF INTRACELLULAR REACTIVE OXYGEN SPECIES-MEDIATED ACTIVATION OF JNK AND NF-κ B, AND AKT INHIBITION
Anthocyanins, phenolic acids, and triterpenoids are bio-active ingredients in a variety of fruits, vegetables, and herbs. These compounds have potent antioxidant activity and have various biological activities that include anticancer activity. However, it is not clear whether these bio-active ingredients can significantly contribute to the protection of embryonic stem (ES) cells from hypoxia-induced apoptosis. This study investigated the effects of a bio-active anthocyanin (delphinidin), phenolic acid (gallic acid), and triterpenoids (betulinic acid and ursolic acid) on hypoxia-induced apoptosis of ES cells. ES cells were incubated in a hypoxic condition for various times. Hypoxia-induced decreases in the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and [^{3}H] thymidine incorporation were evident, but the release of intracellular lactate dehydrogenase (LDH) increased with time. All effects were abrogated by pretreatment with the bio-active ingredients. The effects of the bio-active ingredients on hypoxia-induced reactive oxygen species (ROS) generation were investigated. ROS generation was blocked by pretreatment with all bio-active ingredients in a dose-dependent manner, with the maximum ROS scavenging effect observed for delphinidin. Thus, I examined the protective effect on hypoxia-induced apoptosis of ES cells using delphinidin. Hypoxia increased phosphorylation of c-jun N-terminal kinase (JNK) and nuclear factor-kappa B (NF-κB) were blocked by pretreatment of delphinidin as well as N-acetyl-L-cysteine (NAC). Hypoxia stimulated NF-κB translocation from the cytosol to the nucleus, which was blocked by pretreatment with delphinidin or NAC. Hypoxia decreased the phosphorylation of Akt thr^{308} and ser^{473}; these decreases were reversed by pretreatment with delphinidin or NAC. However, Akt inhibition with pharmacological antagonists did not affect NF-κB phosphorylation. Pretreatment with delphinidin attenuated the hypoxia-induced increase in Bax, cleavage of caspase-3, and decrease in Bcl-2, which also induced by pretreatment of compound SP600125. In addition, the hypoxia-mediated delphinidin-induced decrease in Bax and increase in Bcl-2 were diminished by pretreatment of Akt inhibitor. Hypoxia induced Bax translocation from the cytosol to mitochondria. Furthermore, hypoxia induced mitochondria membrane potential loss and cytochrome c release in cytosol, which were blocked by delphinidin pretreatment. Hypoxia induced cleavage of procaspase-3 which blocked by delphinidin or SP600125 treatment, but Akt inhibitor pretreatment abolished the protection effect of delphinidin. Moreover, inhibition of JNK and NF-κB abolished hypoxia-induced ES cell apoptosis marker expression and inhibition of Akt attenuated delphinidin-induced blockage of apoptosis. The results indicate that delphinidin can prevent hypoxia-induced apoptosis of ES cells through the inhibition of JNK and NF-κB phosphorylation, and restoration of Akt phosphorylation.

T-2324

AN RNAI SCREEN REVEALS TCF-INDEPENDENT FUNCTIONS OF BETA-CATENIN FOR REGULATING PLURIPOTENCY IN MOUSE ESCS

Sujash S. Chatterjee, Ramanuj Dasgupta
Cancer Institute/ Stem Cell Program, NYU School of Medicine, New York, NY, United States

Pluripotency of embryonic stem cells is an essential feature in their potential for use in regenerative medicine. The maintenance of pluripotency in mouse embryonic stem cells (mESCs) is controlled by a transcriptional network regulated by three core transcription factors (TFs), Nanog, Oct4 and Sox2. The balance between pluripotency and differentiation is further influenced by extracellular signals, among which the canonical Wnt/Beta-catenin (Beta-cat) pathway plays a major role in cell fate decisions. Observations that Tcf-3 (a transcriptional effector of Wnt signaling) binds to target regulatory sequences of Oct4 and Nanog, and its loss-of-function results in robust pluripotency, points towards a connection between the Wnt pathway and the activity of the pluripotency maintenance transcriptional network (PTN). Interestingly, Wnt/ Beta -cat signaling has also been shown to promote mesoderm specific differentiation. These observations raise intriguing questions regarding the mechanism(s) by which Beta -cat can coordinate its function in both pluripotency and differentiation. Surprisingly in our RNAi-based functional screen aimed at testing components of the Wnt pathway in mESC pluripotency, we mainly identified components that regulate Beta-cat's stability and its adhesive functions but not the genes involved in modulating Beta-cat's nuclear signaling function. Our results show that small molecules that specifically modulate Beta-cat mediated transcription did not reduce pluripotency markers in cultured mESCs. Additionally, we have found membrane bound complex including Beta-cat, which regulates the balance between self-renewal and differentiation. Differentiation triggers a burst of Wnt/Beta-cat mediated transcriptional activity that coincides with the dis-assembly of the complex.

T-2325

THE ROLE OF PYRUVATE DEHYDROGENASE IN PLURIPOTENCY: CAN WE AFFECT PLURIPOTENCY METABOLICALLY?

Ana Sofia Rodrigues[1], Andreia Gomes[2], Marcelo Correia[1], Tania Perestrelo[1], Joao Ramalho-Santos[1]
[A]Biology of Reproduction and Stem Cell Group, [B]Doctoral Programme in Experimental Biology and Biomedicine (PDBEB), [1]Center for Neuroscience and Cell Biology, Coimbra, Portugal

Pluripotent stem cells are the focal point of a promising field in biomedical research with both advantages and limitations. Similarly to Inner Cell Mass cells and for some types of tumours, embryonic stem cells (ESC) rely mostly on glycolysis for energy supply presenting "silent" mitochondria that, although functional, present a low oxidative phosphorylation activity. On the other hand these cells have high activity of hexokinase II (HK) to favour glucose use, and a phosphorylated (inactive) pyruvate dehydrogenase (PDH) complex to steer pyruvate formed from glycolysis away from the Krebs Cycle, characteristics pluripotent stem cells share with some cancer cells. PDH phosphorylation by one of the four-pyruvate dehydrogenase kinase (PDHK) isoforms leads to PDH complex inactivation, whereas removal of one phosphate group activates this complex. The regulation of PDH activity thus plays an important role in the metabolic flexibility. Although it is known that pluripotent stem cells rely more on glycolysis the exact mechanisms or preferred metabolic pathway is still unknown. In order to better understand the metabolic profile of these cells we are looking to the regulation of the PDH complex and HK in pluripotent vs. differentiated mouse ESCs, with the ultimate goal of manipulating metabolism and therefore pluripotency and differentiation. The PDHK inhibitor Dichloroacetate (DCA) and the HK inhibitor 3-Bromopyruvate (3BP) have both been used in cancer treatment, and we developed an experimental design to determine their effect on pluripotency. We first analyzed cell viability (LIVE/DEAD® Cell viability assay) as well as cell proliferation (Thiazolyl Blue Tetrazolium Blue assay), and found that neither drug affected viability, although there was a decrease in proliferation rates as well as total cell number when compared to the control. Pluripotency was negatively affected, as observed by the decreased staining for alkaline phosphatase as well as in terms of Oct4 and Nanog protein levels. Overall both compounds elevated mitochondrial membrane potential and reactive oxygen species production (accessed by flow cytometry using TMRM and MitoSOX™ Red, respectively). ATP levels were decreased, altering the energetic status of these cells. In addition we observe that in both approaches we have higher lactate levels, but distinct effects on pyruvate levels. We also observed a decrease in GAPDH and HK protein levels. More importantly our cells presented a more active PDH complex in the presence of DCA. A proton NMR spectroscopy approach was used to better characterize these alterations in order to clarify the metabolic prolife of these cells.
Overall our results demonstrate that by pharmacologically manipulating metabolism in specific target enzymes we can induce a shift in pluripotency accompanied with mitochondrial alterations and altered metabolic pathways.

Funding from PTDC/EBB-EBI/101114/2008 and PTDC/QUI-BIQ/120652/2010 grants.

T-2326

NAC1 IS DISPENSABLE FOR STEM CELL MAINTENANCE BUT IS REQUIRED FOR SOMATIC CELL REPROGRAMMING

Francesco Faiola, Miguel Fidalgo, Jianlong Wang
MSSM, New York, NY, United States

Nac1 (Nucleus Accumbens-1) belongs to the Bric-a-brac Tramtrac Broad complex/Pox virus and Zinc finger (BTB/POZ) family of transcription factors. It is ubiquitously expressed and originally identified in the nucleus accumbens of rat brain as a cocaine-inducible gene. Nac1 is also enriched in mouse embryonic stem cells (mESCs), interacts directly with Nanog, and shares many target genes with several stem cells pluripotency factors. Here we show that Nac1 null mESCs can be maintained indefinitely and are pluripotent, as demonstrated by in vitro and in vivo assays. However, loss of Nac1 skews differentiation of mESCs toward the trophectoderm lineage. In addition, somatic cell reprogramming using Nac1 deficient mouse embryonic fibroblasts demonstrates that Nac1 is required for efficient generation of high quality iPSC colonies. The molecular mechanism underlying the distinct function of Nac1 in stem cell maintenance and somatic cell reprogramming is currently unknown and will be discussed in our study.

T-2327

NAIVE-LIKE CONVERSION OF HUMAN EMBRYONIC STEM CELLS THROUGH USE OF SMALL MOLECULES

Margot Van der Jeught[1], Galbha Duggal[1], Dorien Broekaert[1], Sabitri Ghimire[1], Sylvie Lierman[1], Dieter Deforce[2], Tom Deroo[1], Petra De Sutter[1], Björn Heindryckx[1]
[1]Department for Reproductive Medicine, Ghent University Hospital, Ghent, Belgium, [2]Laboratory of Pharmaceutical Biotechnology, Ghent University, Ghent, Belgium

Although human embryonic stem cells (hESC) are derived from the similar blastocyst stage as naive mouse embryonic stem cells (mESC), they show more similarities with primed mouse epiblast stem cells derived from post-implantation embryos. Much current research is focused on the conversion between these different states of pluripotency. Given the distinct differentiation capacities between naive and primed stem cells, the creation of naive human ESC would be of great importance.
Here, we investigated if application of a combination of small molecules, affecting different signalling pathways of interest, can convert existing primed hESC towards a naive, mESC-like state. The small molecule mixture we used was: 3µM Chir99021 (inhibiting GSK3β), 1µM PD0325901 (inhibiting MEK1/2), 10µM forskolin (stimulating adenylate cyclase), and 50ng/mL ascorbic acid. The medium was further supplemented with the growth factors bFGF (12ng/mL) and LIF (1000U/mL). The same formulation was also tested for its capacity to derive mESC-like hESC from blastocyst-stage embryos.
When undifferentiated OCT4-GFP reporter hESC were plated onto MEF feeders in the presence of our small molecule supplemented medium, some dome-shaped colonies were observed 8 to 9 days after plating. The mESC-like dome-shaped colonies were selected manually for further passaging and trypsinization was performed every 4th day. As the passage number further increased, the majority of the colonies started to display a naive, dome-shaped morphology. From this point onwards, passaging of the colonies was performed every 3 days, since we observed an onset of differentiation by the 4th day of culture. These mESC-like colonies could be cultured for at least 20 passages, with continued expression of the OCT4-GFP reporter. At passage 18, qRT-PCR revealed a significant higher expression of genes, known to sustain naive ESC growth. For example, the expression of the naive genes Esrrb, Klf2 and Pecam1 increased 6-fold compared to untreated hESC. Also, a significant decrease in expression of Xist was seen. This may indicate an active X chromosome status, representative of the naive pluripotency state. When the same medium was applied from the blastocyst stage onwards in derivation experiments, several dome-shaped cell clumps emerged after trypsinizing the initial day 6 outgrowths. However, these cells did not survive further passaging. Immunocytochemistry of primary outgrowths revealed the absence of the pluripotency markers OCT4 and NANOG by day 4 post plating in these conditions.
Collectively, our results indicate that the use of chemically defined conditions, targeting different signalling pathways, allows conversion of hESC to adopt mESC-like naive properties. Still, this culture environment was not adequate yet to enable the direct derivation of naive hESC starting from embryos. A better understanding of the molecular pathways that govern early human embryonic development will hopefully inspire new strategies to promote such direct naive derivation.

T-2328

Functional Analysis of NAT1 in Mouse ES Cells

Hayami Sugiyama, Michiyo Koyanagi, Shinya Yamanaka
Department of Reprogramming Science, CiRA,Kyoto Univ, Kyoto, Japan

NAT1 was identified as a candidate RNA modified by the RNA editing enzyme,APOBEC-1, and has amino acid sequence similar to one of the eukaryotic translation initiation factor (eIF) 4G family. eIF4G binds and coordinates cap-dependent translation with eIF4E, eIF3 and eIF4A. Human (h) NAT1 is homologous to the carboxyl two-thirds of eIF4G and binds to eIF3, eIF4A but not to eIF4E. It was also reported that hNAT1 regulates the translation of C-MYC, XIAP, BCL-2, CDK1.
Mouse (m) NAT1 shares high homology (99%) with hNAT1 and is ubiquitously expressed. In our previous study, to analyze the function of NAT1, we generated NAT1-/- mice and NAT1-/- mES cells. While NAT1+/- mice did not show any detectable phenotypic changes, NAT1-/- mice showed embryonic lethality at the gastrulation stage. In addition, NAT1-/- mES cells exhibited an impaired ability to differentiate into cells of all three germ layers and in cell culture, have a rounded, dome-like morphology without differentiated cells. However, the relationship between NAT1 function and those phenotypes is not clear. In this study, we examined whether NAT1 regulates pluripotency in mES cells.
We revealed that NAT1-/- mES cells upregulated several pluripotency-associated genes compared with WT mES cells. After reintroduction of NAT1, rescued mES cells exhibited their gene expression and morphology similar to WT mES cells. In addition, NAT1 was localized in the cytoplasm but not in the nucleus, of mES cells. These results indicate that NAT1 influences transcription of the pluripotency-associated genes indirectly through from the cytoplasm. We are investigating NAT1-binding targets which are related to the pluripotency of mES cells.

T-2331

A NOVEL REGULATORY FACTOR FOR THE PROLIFERATION AND LINEAGE-SPECIFIC DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS IN MITOCHONDRIA

Yuko Kokubu[1], Megumi Kowno[1], Kanako Watanabe-Susaki[2], Hisako Ishimine[4], Shinji Komazaki[6], Yasuhiro Seki[7], Youhei Ishigaki[1], Naoto Ninomiya[7], Taka-aki K. Noguchi[1], Yoshiro Nakajima[3], Pi-Chao Wang[1], Makoto Asashima[8], Akira Kurisaki[5]
[1]Graduate School of Life and Environmental Sciences, The University of Tsukuba, Tsukuba, Ibaraki, Japan, [2]National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan, [B]Research Center for Stem Cell Engineering, [2]National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan, [3]The University of Tsukuba and National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan, [B]Graduate School of Life and Environmental Sciences and Research Center for Stem Cell Engineering, [3]The University of Tsukuba and National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan, [4]Department of Anatomy, Saitama Medical School, Saitama, Japan, [5]Department of Life Sciences (Biology), Graduate School of Arts and Sciences, The University of Tokyo, Meguro, Tokyo, Japan, [6]National Institute of Advanced Industrial Science and Technology (AIST), The University of Tokyo and The University of Tsukuba, Ibaraki and Tokyo, Japan

The pluripotent state of embryonic stem (ES) cells is controlled by a transcription network composed of specific transcription factors. Recent studies also suggested the significant contribution of cellular metabolism on the proliferation and differentiation of pluripotent stem cells. However, the molecular basis of these regulations is still not well understood. In this study, we found that prohibitin 2 (PHB2), a pleiotrophic factor mainly localized in mito-

chondria, is a crucial regulator of the homeostasis and differentiation of ES cells. PHB2 was highly expressed in undifferentiated mouse ES cells, and the expression was decreased during the differentiation of ES cell. Knockdown of PHB2 induced apoptosis in pluripotent ES cells, and enhanced expression of PHB2 contributes to the proliferation of the undifferentiated state of ES cells. However, sustained expression of PHB2 strongly inhibits ES cell differentiation. Interestingly, the mitochondrial targeting signal-mutated PHB2 failed to show these specific effects on ES cells, indicating that mitochondrial PHB2 plays crucial regulatory functions in ES cells. Our results suggested that mitochondrial PHB2 is a crucial regulatory factor for the homeostasis and lineage-specific differentiation of ES cells.

T-2332

THE ROLE OF MICRORNAS IN HUMAN EMBRYONIC STEM CELL SURVIVAL

Cullen Pivaroff[1], Heather Schultheisz[2], Francesca Boscolo[3], Jeanne Loring[2], Louise Laurent[4]
[1]Biology, San Diego State University/ The Scripps Research Institute, San Diego, CA, United States, [2]Department of Chemical Physiology, The Scripps Research Institute and the Center for Regenerative Medicine, San Diego, CA, United States, [3]Department of Chemical Physiology, Department of Reproductive Medicine, The Scripps Research Institute and the Center for Regenerative Medicine, University of California, San Diego, San Diego, CA, United States, [4]Department of Reproductive Medicine, University of California, San Diego, San Diego, CA, United States

MicroRNAs (miRNAs) are short non- coding RNAs that have been shown to post-transcriptionally regulate gene expression in animal cells. It is believed that miRNAs negatively regulate gene expression by interacting with messenger RNA (mRNA) in the context of an RNA-induced silencing complex and promoting either translational repression or mRNA degradation. There is evidence that miRNAs are vital to many processes including development, the cell cycle, cell survival, and apoptosis, and that dysregulation of miRNA expression can play a role in cancer. It has been suggested that each cell type and tissue expresses a unique and characteristic combination of active miRNAs. Although there is substantial published evidence that miRNA families highly expressed in hESCs can be used to reprogram somatic cells to pluripotency, the mechanisms through which these miRNAs act in the establishment and maintenance of the pluripotent state are not fully understood. It is known that these miRNAs play a critical role in the self-renewal and cell-cycle characteristics of pluripotent stem cells, but potential effects on other pathways remain to be identified.
In preliminary experiments, we have observed that knockdown of miRNAs hsa-miR-302a and hsa-miR-525-5p resulted in decreased hESC survival through increased apoptosis. In this project, we seek to identify the target mRNAs through which these two miRNAs are acting to suppress apoptosis in hESCs. We have used miRNA target prediction algorithms to identify candidate targets that are associated with apoptosis. We are using luciferase reporters, qRT-PCR, and Western blot analysis to determine which of these candidates are functionally repressed by hsa-miR-302a and hsa-miR-525-5p, and the mechanism by which the repression is occurring. Future studies will be directed at evaluating the regulation of these targets during differentiation, and whether manipulation of these targets can be used to improve survival of human pluripotent cells both in the undifferentiated state and during directed differentiation.

Embryonic Stem Cell Differentiation

T-2333

GENOME ORGANIZER SATB1 REGULATES DIFFERENTIATION-ASSOCIATED SILENCING OF THE NANOG LOCUS BY MODULATING ITS NUCLEAR POSITIONING IN HUMAN EMBRYONIC STEM CELLS

Mohammed Ahmed[1], Neil J. Harrison[2], Dylan Stavish[2], Christian Unger[2], Andrei N. Mardaryev[1], Michael Y. Fessing[1], Krzysztof Poterlowicz[1], Peter W. Andrews[2], **Vladimir A. Botchkarev**[3]

[1]Centre for Skin Sciences, Univ of Bradford, Bradford, United Kingdom, [2]Centre for Stem Cell Biology, Univ of Sheffield, Sheffield, United Kingdom, [3]Centre for Skin Sciences/Dept Dermatology, Univ of Bradford/Boston Univ, Boston, MA, United States

During development, gene expression programmes in multi-potent stem cells are re-organized at several levels including higher-order chromatin remodelling and nuclear compartmentalization of the genomic loci and transcription machinery. We have analyzed changes in three-dimensional organization of the Nanog gene locus during differentiation of the human embryonic stem cell line H14 in culture. Stem cell differentiation was monitored by downregulation of expression of stem cell markers SSEA-3 and TRA-1-60 in cultured cells. By microarray and RT-PCR analyses, expression of the Nanog gene was strongly decreased in differentiating SSEA-3 negative cells, compared to SSEA-3 positive stem cells. These changes were accompanied by significant changes in the intra-nuclear Nanog gene positioning, as determined by 3D-FISH analyses and confocal microscopy. In differentiating cells, the Nanog gene locus was located more peripherally relatively to the chromosome territory 12, compared to undifferentiated stem cells, in which the Nanog locus was located more internally. Genome organizer Satb1 protein showed higher expression in differentiating cells, compared to undifferentiated stem cells. Furthermore, shRNA-mediated Satb1 knockdown resulted in the retention of the Nanog gene in the nuclear interior, associated with increase of its transcriptional activity, compared to controls. Thus, these data demonstrate that Satb1 regulates differentiation-associated silencing and relocation of the Nanog locus from the nuclear interior towards nuclear periphery and suggest that higher-order chromatin remodelling is a key regulatory mechanism required for a proper control of cell differentiation process in multi-potent stem cells.

T-2334

EPIGENETIC EFFECTS OF ALCOHOL ON MOLECULAR SIGNATURES IN HUMAN STEM CELLS

Omar Khalid, Jeffrey Kim, Hyun-Sung Kim, Connie Lee, Catherine Vu, Steve Horvath, Igor Spigelman, Songtao Shi, Yong Kim
Dentistry, UCLA, Los Angeles, CA, United States

Objective: Alcohol abuse has been shown to lead to a variety of injuries in embryonic development as well as in adult life. Alcohol consumption has led to detrimental affects such as intrauterine growth retardation, nervous system damage, triggers for carcinogenesis in both oral and hepatic health, as well as deleterious damage to the individual as a whole. We hypothesize that these affects may stem from how alcohol can change genetic and epigenetic regulation of cells when they are introduced to alcohol.
Methods: We used hESCs (line H9) to answer the fundamental questions as to the effect of ethanol (EtOH) on the molecular signatures in stem cell populations in an unbiased manner. We examined three conditions: 1) Undifferentiated hESCs treated with EtOH, 2) hESCs differentiated to embryoid bodies (EB) and subsequently treated with EtOH, and 3) the basic process of differentiation from an undifferentiated stem cell to EBs. To gain insight into the genetic mechanisms of EtOH-induced alterations in stem cells we examined changes in their differentiation potential upon EtOH treatment and how the genes behaved as groups. For gene expression and DNA methylation microarray analyses, we used Weighted Gene Co-Expression Network Analysis (WGCNA) and annotated with the R package cluster Profiler.
Results: Utilizing hESCs, we examined how EtOH can alter the genome as well as the methylome. We saw striking changes in gene profiles for hESCs treated with EtOH either in an undifferentiated and differentiated setting. These changes affected metabolic and neuronal like activities of stem cells. Furthermore, when examining the methylome we observed significant changes occurring on “hot spots” of the genome including regions of Chromosome 1, 19 and the X chromosome. We also correlated our methylation and genetic results and found that treatment with 5-AZA had the potential to reverse some of the effects observed.
Conclusion: Our results demonstrate how alcohol functions in altering stem cell behavior leading to a more differentiated altered state epigenetically. In certain settings these changes can be reversed when using epigenetic altering drugs. We anticipate these findings to be the initial step for further studies to identify the effects of alcohol in altering the genetic and epigenetic landscape. These in turn may be related to specific sequence elements and

transcriptional on/off switches critical in gene regulation. Identifying these switches within this context can help us in treatment of alcohol-related diseases.

T-2335

FOXA2 ACTS AS A CO-ACTIVATOR TO POTENTIATE NURR1-INDUCED DA PHENOTYPE GENE EXPRESSION VIA AN EPIGENETIC REGULATORY MODE

Xi-Biao He, Sang-Hoon Yi, Yong-Hee Rhee, Sang-Hun Lee
Hanyang University, Seoul, Korea, Republic of

Understanding how dopamine (DA) phenotypes are acquired in midbrain DA (mDA) neuron development is an important issue, as it can be utilized in stem cell manipulations for the treatment of mDA neuron-associated disorders such as Parkinson's disease. Nurr1, an orphan nuclear receptor, is known as a critical transcription factor for DA phenotype gene expressions. However, it alone exerts insufficient functions and requires other co-activators. In this study, we demonstrate a feedforward manner of mDA neuron development, in which Foxa2, expressed from early developing ventral midbrain (VM), cooperates with Nurr1 for the acquisition of DA phenotype gene expressions in late VM neural precursor cells (VM-NPCs). Co-localization of Nurr1 and Foxa2 expressions in the precursors were established by a positive cross regulatory loop between these factors, and DA phenotype gene expressions were acquired exclusively in the VM-NPCs co-expressing Nurr1 and Foxa2. Multiple lines of experiments revealed that Nurr1 and Foxa2 were co-occupied at the promoter regions of the DA phenotype genes TH and DAT, via forming a physical Nurr1-Foxa2 protein complex. In the absence of Foxa2, Nurr1 proteins bind to CoREST, a common epigenetic repressor. The inhibitory role of CoREST in Nurr1-mediated DA gene transcriptions was mediated by recruiting HDAC1, an enzyme catalyzing histone deacetylation, to the DA gene promoters. In the presence of Foxa2, the interaction of Nurr1-CoREST was diminished (probably by competitively forming Nurr1-Foxa2 activator complex), and CoREST/HDAC1 proteins were less enriched in the DA gene promoters. Consequently, histone 3 acetylation (H3Ac), a chromatin code responsible for open chromatin structures, was strikingly increased at multiple regions of TH and DAT promoters. These findings strongly suggest Foxa2 and Nurr1 interact together and epigenetically activate DA gene transcriptions during the mDA neuron development.

T-2336

PROBING PLURIPOTENT STEM CELL PRE-PATTERNING DYNAMICS WITH RAMAN SPECTROSCOPY

James E. Mason[1], Wei Huang[2], Katherine Lau[3], Peter W. Andrews[1]
[A]Biomedical Science, [B]Civil and Structural Engineering, [1]University of Sheffield, Sheffield, United Kingdom, [2]Renishaw Plc, Wotton-under-Edge, United Kingdom

It is well established that apparently uniform pluripotent stem cell populations can generate a variety of derivatives when exposed to a differentiation stimulus. The mechanism(s) by which this phenomenon occurs are not well characterised. One possibility is that stem cells may exist in different inter-convertible substates that have differential lineage bias prior to differentiation induction. For example, NTERA2.D1, a pluripotent human embryonic carcinoma cell line appears to exist in pro-neural and non-neural substates that are revealed when the cells are induced to differentiate by retinoic acid exposure (Tonge et al, 2010). However, a marker prospectively identifying these substates prior to differentiation has not yet been identified.
Common approaches to identifying such substates include the use of surface antigens (Tonge et al, 2011), or of reporter genes, but these have the drawback of potentially interfering with the cells and the dynamics of the substates they occupy in unknown ways. An alternative is to use Raman spectroscopy to record the biochemical profiles of individual, live cells, thus potentially enabling the identification of substates with minimal interference. Various cell tracking or sorting approaches could then be used to assess the behaviour and possible lineage bias of stem cells occupying substates identified in this way.
To validate the use of Raman spectroscopy in the context of probing pluripotent stem cell behaviour we have used both unsupervised and supervised statistical clustering methods to establish whether it is able to distinguish suffi-

ciently between pluripotent stem cells known to possess phenotypic differences. Raman signatures were compared between different stem cell lines, different stages of retinoic acid induced differentiation, and different sub-cellular regions. We were able to establish that Raman spectroscopy is sensitive enough to distinguish between a variety of both human embryonic carcinoma and human embryonic stem cells, and their retinoic acid induced differentiated derivatives. The differences in Raman signature between undifferentiated and retinoic acid treated NTERA2.D1 cells were detectable as little as 3hrs post-induction. In addition, differences in Raman signatures from subcellular nuclear, nucleolar and cytoplasmic sub-cellular biochemical profiles were observed; all of which are known to change during differentiation.
Our results indicate that Raman spectroscopy can be used to distinguish subtle differences in pluripotent cell phenotypes and offers significant potential to uncover biochemical markers of functional pre-patterning in the stem cell compartment.

T-2337

PREDICTING DEFINITIVE EDODERM DIFFERENTIATION POTENTIAL OF HUMAN PLURIPOTENT STEM CELL LINES

Wei Jiang, Yi Zhang
Harvard Medical School, Boston Children's Hospital, Boston, MA, United States

Human pluripotent stem cells, including hESCs and iPSCs, are promising cell sources for cell therapy and drug screening due to their capacity for unlimited self-renewal as well as pluripotency. Currently, various in vitro differentiation protocols have been developed for generation of the different functional cell types. However, each of these differentiation protocols is typically developed based on one or a limited number of hESC lines and it may not be applicable to other cell lines. Indeed, different hESC and iPSC lines exhibit substantial variation in their capacity in differentiating into different cell lineages. Recently, a genome-wide gene expression analysis of a large collection of hESC and iPSC lines have generated a scorecard based on the expression level of about 500 lineage-specific genes. However, this method is technically complicated and not economical for routine laboratory studies. Thus, a simple and economical experimental approach for accurate prediction of hESC and iPSC differentiation potential is needed. In addition, a recent study demonstrated that the expression of miR-371-3 cluster could predict neural differentiation propensity in human pluripotent stem cells, which suggests that lineage-specific differentiation potential can be predicted using simple biomarkers. Here we utilized a well-established definitive endoderm differentiation system, which has also been shown working very well in our lab, and established WNT3 as a biomarker capable of predicting hESC differentiation potential for definitive endoderm. We have observed that the mRNA level of WNT3 in hESCs correlates with definitive endoderm differentiation potential when hESC lines are subjected to a well-established differentiation protocol. In addition, manipulations of hESCs through WNT3 knockdown or overexpression can respectively inhibit or promote endoderm differentiation in a WNT3 level-dependent manner. Finally, the correlation between WNT3 expression level in hESCs and definitive endoderm differentiation efficiency is still observed under different definitive endoderm differentiation protocols. Collectively, our data supports that WNT3 can serve as a biomarker for predicting definitive endoderm differentiation potential of hESCs.

T-2338

FUNCTIONAL INVESTIGATIONS OF THE BMP INHIBITOR NBL1 DURING CONTROL OF LIVER/PANCREATIC FATE SPECIFICATION FROM PLURIPOTENT STEM CELLS

Carolina Parada, Michael Bukys, Jan Jensen
Stem Cell Bilogy, Clevelan Clinic Foundation, Cleveland, OH, United States

FUNCTIONAL INVESTIGATIONS OF THE BMP INHIBITOR NBL1 DURING CONTROL OF LIVER/PANCREATIC FATE SPECIFICATION FROM PLURIPOTENT STEM CELLS
Parada, Carolina., Bukys, Michael., and Jensen, Jan.
Abstract:

Bone morphogenetic proteins (BMPs) are multi-functional growth factors that belong to the transforming growth factor beta (TGFbeta) superfamily. They provide specific signaling for specifying embryonic patterning, organ development and tissue regeneration. BMP signaling is essential for hepatic specification allowing bi-potential ventral pancreatic/hepatic progenitor cells in the endoderm layer to induce liver gene expression in response to FGFs. As a result, this inhibits the cellular differentiation into pancreatic cells, thus inducing liver fate. BMPs have been shown to be detrimental to the generation of pancreatic fates especially in *in vitro* human embryonic stem (hES) model systems. Inhibition of the BMP signaling pathway with the exogenous protein Noggin at specific stages of directed differentiation protocols have positively affected the production of pancreatic cells, and also improved pancreatic insulin cell formation. However, those protocols have not succeeded in the complete inhibition of liver development instead obtaining a diverse population of cells which include few immature β-cells, glucagon positive cells, and functional hepatocytes. While the BMP signaling inhibitor Noggin has been used in directed differentiation of hESC towards pancreas, *Noggin* is unlikely to be a physiological inhibitor in the embryo at this transition. In search for candidate BMP-inhibitors, we performed genomics analysis of pancreatic mesenchyme revealing the presence of the BMP antagonist *Nbl1*. This was confirmed with immunochemistry staining embryonic mouse pancreas. The capacity of Nbl1 to act as a Bmp antagonist was validated by its ability to inhibit BMP-induced expression of Id1,2,3 and 4 levels in a Hek-293 cell based assay. We next evaluated the role of Nbl1 during hESC forward differentiation to definitive endoderm and liver/pancreas. Nbl1 was comparable to Noggin in inhibition of hepatic fate using a directed differentiation protocol. We also demonstrated effective inhibition of Id-factor expression in hESCs, additionally corroborated by western blot analysis of phospho-smads 1/5/8, which was also inhibited. Immunocytochemical analysis of pSmads 1/5/8 in cells treated with BMP4 plus Nbl1 also demonstrated loss of nuclear phosphorylated-SMAD. These data reveal that the use of Nbl1 can effectively down-regulate *ALB+* cell expression especially at stages 2-3 of the directed differentiation protocol as observed in protein level and transcript level analysis. Moreover, Nbl1 was permissive for definitive endoderm induction and did not interfere with the up-regulation of definitive endodermal genes, even when provided at time of Definitive Endoderm induction. While still needed to be validated in the context of normal pancreatic development, these findings indicate that the BMP inhibitor Nbl1 is perhaps a more physiologically-relevant endogenous BMP antagonist that operates during pancreatic differentiation.

T-2341

CDK1 IS REQUIRED FOR PLURIPOTENCY MAINTENANCE AND DOWN-REGULATION OF CDK1 PROMOTES DIFFERENTIATION OF HUMAN EMBRYONIS STEM CELLS

Xiao Qi Wang[1], Lin Chen[1], Chris Norbury[2]

[1]Dept. of Surgery, Univ of Hong Kong, Hong Kong, China, [2]Sir William of Dann School, Univ of Oxford, Oxford, United Kingdom

Embryonic stem cells (ESCs) and induced pluritpotent stem cells (iPSCs) are invaluable tools for the study of developmental biology, understanding and modeling disease, drug discovery, and stem cell-based therapies, which needs comprehensive understanding of the mechanisms of the regulatory of pluripotency, lineage-specific differentiation, and cellular reprogramming. Appropriate cell cycle regulation may be critical for pluripotency and the definition of cell fate, accumulating evidence indicates that the role of cell cycle regulators in control of pluripotency and differentiation goes beyond their role as cell cycle effectors. Cyclin-dependent kinase 1 (CDK1) is one of the most pleiotropic core cell cycle regulators; it is also indispensable during embryonic development and predicted to potentially regulate a large number of substrates during stem cell differentiation.
In this study, we investigated the potential role of CDK1 in regulation of pluripotency and differentiation. Inactivation of CDK1 by a selective and reversible CDK1 inhibitor (RO-3306) promoted human ESCs (H7 & H9 lines) differentiation and negative or reduced Alkaline Phosphotase (AP) staining. RO-3306 treatment also resulted in: (a) reduced expression of pluripotency markers OCT4 and SSEA4 at the protein level and OCT and NANOG at the transcriptional level; (b) up-regulation of mesendoderm transcripts BRACHYURY, EOMES, and MIXL1; (c) EMT phenomena such as decreased epithelial marker E-CADHERIN and enhanced expression of the mesenchymal marker SLUG. The differentiation did not seem to associating with the cell cycle arrest; as under the conditions used, RO-3306

was not sufficient to block hESCs in G2 cell cycle. Genetic knock-down of CDK1 expression in hESCs using shRNA (shCDK1) significantly decreased AP positive hESC colonies compared to shControl, indicating an self-renewal inhibition. shCDK1 also promoted down-regulation of the pluripotency marker OCT4, to a similar extent as serum-induced differentiation. Interestingly, knocking down CDK1 in hESCs led to an apparent decrease in Akt phosphorylation (Ser473 and Thr308), whereas phosphorylation of Erk and Gsk3β were remained unchanged. Moreover, a low dose of JNJ-770621, a dual inhibitor of CDK1/CDK2 and Aurora kinases but not Akt or Erk, induced human embryonal carcinoma cell (NCCIT) differentiation accompanied by reduced phosphorylation of Akt. Although high dose RO-3306 could inhibit Akt or Erk kinases, the low dose (8-10 [[Unsupported Character - Symbol Font ]]M) we used for differentiation did not suppress or alter phosphorylation of Akt or Gsk3[[Unsupported Character - Symbol Font ]]. The results suggest that level of CDK1 might cross-regulate key signaling pathways such as that operating through PI3K-Akt in regulation of pluripotency and differentiation.
In summary, CDK1 is required for maintenance of stem cell pluripotency, in a manner that is distinct from its role in regulation of cell cycle progression, through direct or indirect cross-regulation of fundamental pluripotency signaling including PI3K/Akt pathway.

T-2342

THE ROLE OF CDX2 EXPRESSION IN BMP4-INDUCED TROPHOBLAST DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

Yasunari Seita, Chi-Wei Lu
OB/GYN, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, NJ, United States

The unique requirement for Cdx2 in the development of mouse placental trophoblasts has been well-studied, in genetic models and through manipulating its expression in embryonic stem cells. The role of CDX2 in human trophoblast differentiation remains elusive. We studied the involvement of CDX2 in trophoblast differentiation from human embryonic and induced pluripotent stem cells (ESCs and iPSCs). From BMP4-induced trophoblast differentiation, we observed that CDX2 expressed in nearly 100% of cells, and largely overlapping with OCT4 expression, suggesting the model of their mutual transcription repression seen in the mouse is not conserved in humans. We tested whether CDX2 expression alone is associated with a trophoblast progenitor fate, through generating iPS cell lines with ectopic CDX2 expression. Two CDX2 expressing cell lines were analyzed, both expressing telomerase activity and capable of differentiation into syncytiotrophoblast after switching into serum containing media. However, in the cell line expressing CDX2 at a very high level, differentiation into mesoderm and endoderm lineages was also observed, suggesting the effect of CDX2 for trophoblast differentiation is dose dependent. Repressing CDX2 expression by shRNA does not blockade BMP4-induced trophoblast differentiation. Altogether, our results suggest that while the transcription regulation is different from the mouse, CDX2 in the human plays a conservative role in trophoblast development as being not necessary for differentiation, but sufficient for inducing a trophoblastic-stem cell like phenotype from pluripotent stem cells.

T-2343

NOVEL MECHANISM FOR VERTEBRATE BONE DEVELOPMENT: MICRORNA 361 PROMOTES NEURAL CREST FORMATION VIA PRICKLE-1 DURING OSTEOGENIC DIFFERENTIATION OF ESCS

Dorota Kaniowska[1], Sung Seo[2], Nicole zur Nieden[2]
[1]*Translational Center for Regenerative Medicine Leipzig/Germany, Leipzig, Germany,* [2]*Department of Cell Biology and Neuroscience and Stem Cell Center, College of Natural and Agricultura, University of California Riverside, Riverside, CA, United States*

Objective: A unique feature of vertebrate embryonic development is the formation of the skeletal system, which provides mechanical support and mineral balance for the organism. Skeletal precursor cells give rise to either chondrocytes to form cartilage or osteoblasts to build bone. Cartilage and bone both develop from mesenchyme derived from the neural crest (NC), paraxial mesoderm, or lateral plate mesoderm. Bone in contrast to cartilage also

possesses the intrinsic capacity for regeneration as part of the repair process during skeletal development as well as in response to injury, or continuous remodeling throughout adult life. Osteoblast differentiation is a key step in proper skeletal development and acquisition of bone mass. All of the major developmental signals including Wnt and Notch signaling, along with an increasing number of transcription factors such as Runx-2 and osterix, have been shown to regulate the differentiation and/or function of osteoblasts. However, the regulation of osteogenic cell fate decisions by microRNAs (miRNAs, miRs) and their use to turn stem cells or progenitors into osteoblasts has been reported, but remains inefficient. In order to elucidate the role of miRNAs in osteogenesis, we have turned to murine embryonic stem cells (mESCs), which are an ideal model to study the earliest events of pre-osteogenic specification. Using this model, we focused on identifying lineage committing miRNAs, which are known molecular regulators of developmental processes. **Material and methods:** In a screen using a special miRNA array, we found altered miRNA levels following spontaneous differentiation and vitamin D3 induced osteogenic differentiation of mESCs. miRNA profiling showed up- and down-regulation of miRNAs that have predicted targets involved in embryonic development and bone formation. We have identified a miRNA - miR-361 - that showed differential expression. Overexpression and knock-down studies were then coupled with assessment of calcification potential and expression of osteoblast markers to determine whether miR-361 promoted osteoblast differentiation. Furthermore, bioinformatical predictions of miR-361 targets were experimentally followed to identify its mRNA target in osteogenesis using mRNA expression analysis upon overexpression and knockdown and RNAi strategies. **Results:** Overexpression and knockdown of miR-361 in mESCs caused changes in calcium accumulation and osteogenic marker gene expression. Furthermore, miR-361 overexpression resulted in a reduction of mRNA levels of Prickle-1, which is a core component of the Wnt/planar cell polarity pathway and a nuclear translocator receptor for RE-1 silencing transcription factor. RNAi and Western blot assays further confirmed that Prickle-1 is a direct target of miR-361. Moreover, in order to demonstrate that miR-361 presence has an influence on NC development, we performed PCR analysis of Sox-1 and Sox-10. Enhanced levels of miR-361 resulted in significant increases of both markers. **Conclusions:** Assuming that miR-361 plays a functional role in modulating osteogenic differentiation of ESCs we are indicating new mechanisms involved in this complex regulatory scheme. Unraveling the regulatory circuits of miRNAs is a great challenge, but may provide attractive targets for mechanism-based treatment of bone diseases.

T-2344

MICRORNAS AS NETWORK LEVEL ORCHESTRATORS MODULATING NEURAL CONVERSION OF HUMAN EMBRYONIC STEM CELLS

Zhenghua Sun, Yi Huang, Jing-Dong J. Han
CAS-MPG Partner institute for Computational Biology, shanghai, China

MicroRNAs (miRNAs) are powerful gene expression regulators by targeting most human genes. A single miRNA can target many genes, and multiple miRNAs can target the same gene. This mode of function suggests that miRNAs act on “network-level” modulating complex biological processes by integrating multiple inputs and outputs, e.g. neural conversion of human embryonic stem cells (hESCs). Neural stem cells could be regarded as quasi-intermediate state in neural conversion of hESCs with self-renewal ability and limited multipotency to differentiate neurons and glia cells, which is an available model on the studies of human neurogenesis and neurological diseases. Classical neural induction protocol of hESCs rely on formation of embryoid bodies, which is a process of spontaneous differentiation and their heterogeneous nature leading to a break on tracing the conversional trajectories. Based on a method by dual inhibition of SMAD signaling, we have established a continuous unidirectional neural differentiation system of hESCs. More than 90% cells are identified as neural stem cells by detecting Nestin expression and the purity increased to nearly 95% after cultured in EGF/bFGF maintain medium. Then we performed miRNA profiling using Solexa sequencing on key time points during neural induction. As expected, miRNA expression patterns shift significantly in a continuous range. Stage-specifically expressed miRNAs were identified as a miRNA modules. Target genes analysis for these modules revealed that they regulate differentiation pathways in a chronological order, such as hedgehog signaling pathway in early stage and notch signaling pathway in later period. All of this indicated that miRNAs might act as orchestrators on genetic network level modulating neural conversion of human embryonic stem cells.

T-2345

MIR-27 SUPPRESSES SELF-RENEWAL IN HUMAN EMBRYONIC STEM CELLS AND EMBRYONAL CARCINOMA CELLS

Heiko R. Fuchs, James Adjaye
Institut für Stammzellforschung und regenerative Medizin, Duesseldorf, Germany

Human embryonic stem (hES) and embryonal carcinoma (hEC) cells have been studied extensively with respect to the transcription factors (OCT4, SOX2 and NANOG), epigenetic modulators and associated signalling pathways that either promote self-renewal or induce differentiation in these cells1,2,3. The ACTIVIN/NODAL axis (SMAD2,3) of the TGFß signalling pathway coupled with FGF signalling maintains self renewal in these cells, whilst the BMP (SMAD1,5,8) axis promotes differentiation. We previously demonstrated that FGF2 induces the expression of TGFß ligands like GREM1, TGFß1, and INHBA in hES cells to sustain self renewal4. MicroRNAs (miRNAs) play a critical role in a variety of biological processes including development, proliferation, cell growth and lineage commitment. Recently, embryonic specific miRNAs have emerged as crucial regulators of self renewal and pluripotency. Recent studies demonstrate that somatic cells can be reprogrammed to induced pluripotent stem cells (iPS) by lentiviral transduction of embryonic specific miRNAs5. In the quest to derive integration-free iPS cells we aim at a reprogramming approach incorporating over-expression of embryo-specific miRNAs and simultaneous inhibition of a selection of somatic-specific miRNAs using antagonists.
We focused on miR-27, a somatic-specific miRNA, we show that miR-27 is activated upon RNAi-mediated suppression of OCT4 function in ES and EC cells. Employing GFP-based sensor constructs we further demonstrate that miR-27 negatively regulates the expression of the pluripotency-associated genes, OCT4, NANOG, LIN28, TGFßR1, POLR3G and NR5A2, an established upstream regulator of OCT4 expression6. In summary, our data indicate that miR-27 expression might act as a barrier to the induction of pluripotency in somatic cells.

T-2346

WNT-REGULATED MICRORNAS CONTROL LINEAGE DIFFERENTIATION IN MOUSE EMBRYONIC STEM CELLS

Yaser Atlasi[1], Rubina Noori[1], Claudia Gaspar[1], Patrick Franken[1], Andrea Sacchetti[1], Tokameh Mahmoudi[1], Charles Decraene[2], Leendert Looijenga[1], George Calin[3], Bradley Merrill[4], Ricardo Fodde[1]
[1]Erasmus MC, Rotterdam, Netherlands, [2]4CNRS, Paris, France, [3]MD Anderson Cancer Center, Texas, TX, United States, [4]University of Illinois, Chicago, IL, United States

Canonical Wnt signaling plays a rate-limiting role in regulating self-renewal and differentiation in mouse embryonic stem cells (ESCs). We have previously shown that mutation in the *Apc* (adenomatous polyposis coli) tumor suppressor gene constitutively activates Wnt signaling in ESCs and inhibits their capacity to differentiate towards ecto-, meso- and endodermal lineages. However, the underlying molecular and cellular mechanisms through which Wnt regulates lineage differentiation in mouse ESCs remains to date largely unknown.
To this aim, we have derived and studied the gene expression and microRNA profiles of *Apc*-mutant ESC lines encoding for different levels of Wnt signalling activation. We report here the identification of several novel Wnt-regulated miRNAs (e.g mmu-miR-211) in *Apc*-mutant mouse embryonic stem cells, which were subsequently validated by transient activation of Wnt signalling in wild-type ESCs using Wnt3a and GSK-inhibitor treatment. By employing DOX-inducible or constitutive overexpression in wild type and *Apc*-mutant ESCs followed by *in vivo* (teratoma formation) and *in vitro* differentiation assays, we found that Wnt-regulated miRNAs directly contribute to different degrees of the differentiation defects observed in *Apc*-mutant ESCs. We show that the Wnt-induced microRNA, miR-211, directly targets Tcf3 (a member of Tcf/Lef family and a key player in the control of self-renewal and pluripotency) and attenuates early neural differentiation in mouse ESCs. Expression of *Tcf3* in Apc-mutant ESCs could partially rescue the neural defects observed in these cells.
Our data provide a more detailed picture of the downstream effects of Wnt signalling and highlight novel mechanisms through which Wnt signalling inhibits neuro-ectodermal lineage differentiation in mouse embryonic stem cells.

T-2347

STUDYING THE ROLE OF MIRNAS IN THE FUNCTION AND DYSFUNCTION OF DOPAMINE NEURONS DERIVED FROM STEM CELLS

Woori Kim[1], Yenarae Lee[1], Arthi Shanmugavadivu[1], Donna McPhie[1], Kwang-Soo Kim[2], Bruce Cohen[1], **Kai C. Sonntag**[1]

[A]Psychiatry, Frazier Institute, [B]Psychiatry, [1]McLean Hospital, Harvard Medical School, Belmont, MA, United States

miRNAs are short non-coding RNAs that regulate gene expression at the posttranscriptional level. They are involved in cellular homeostasis and have been implicated in normal aging and in disease pathogenesis, including in the mammalian brain. miRNAs could be important factors in therapy development because they represent targets for “smart molecules” that can be designed to alter gene expression in a process and cell-specific manner to correct disturbed cell functions. We recently identified two miRNAs, miR-126 and miR-320, that were upregulated in laser-captured postmortem substantia nigra (SN) dopamine (DA) neurons from sporadic Parkinson Disease (PD) patients and aged-matched controls. Both miRNAs are associated with regulation of Insulin/IGF-1, PI3K, MAPK, and AKT signaling and they have been implicated in vascular and heart disease, cancer, inflammation, and diabetes. Dysfunctional Insulin/IGF-1/PI3K signaling has also been linked to aging and PD pathogenesis, and bioinformatics analysis on our miRNA/mRNA profiles from the postmortem DA neurons generated correlative evidence for an association of miR-126 and miR-320 with this pathway in these cells. To determine a role of these miRNAs in the function or dysfunction of DA neurons, we generated lentivirus vectors that specifically and conditionally express miRNAs in DA neuronal cell systems, including SH-SY5Y neuroblastoma cells, embryonic ventral mesencephalon cultures, and human embryonic stem cell (hESC)-derived DA neuronal lines. These studies showed that overexpression of miR-126 and miR-320 increased sensitivity to toxic stress induced by 6-hydroxydopamine (6-OHDA) and impaired a neuroprotective effect of IGF-1. In the case of miR-126 this effect was associated with downregulation of factors in the Insulin/IGF-1/PI3K signaling cascade, including phosphorylated AKT and ERK, and the miR-126 targets IRS-1, p85β, and SPRED1. Our data provide evidence that miR-126 and miR-320 may convey vulnerability to toxic insult in DA neurons by deregulating factors in the IGF-1/PI3K signaling pathway. Stem cell-derived model systems of neurogenesis may provide a tool to study the regulation of signaling cascades by miRNAs in both normal and pathological conditions. This approach may have implications for understanding the molecular mechanisms of aging and disease as they affect neurons and may aid in the design of future therapies for age-related brain disorders.

T-2348

NOTCH1 ACTS VIA FOXC2 TO INDUCE THE ENDOTHELIAL TO HEMATOPOIETIC TRANSITION DURING EMBRYONIC DEVELOPMENT

Yi-Fen Lu, Il-Ho Jang, George Daley

Hematology/Oncology, Children's Hospital Boston, Boston, MA, United States

Hematopoietic and vascular development share many common features, including cell surface markers and sites of origin. Recent lineage tracing studies have established that definitive hematopoietic stem cells (HSCs) arise from VE-Cadherin+ hemogenic endothelial cells of the aorta-gonad-mesonephros (AGM) region, but the genetic programs underlying this endothelial to hematopoietic transition remain undefined. Here, we observed that Notch induction dramatically enhances hematopoietic potential in differentiating cultures of mouse embryonic endothelial population. Studies in zebrafish and mouse embryos indicated that FoxC2 and its orthologs are downstream of Notch signaling in hemogenic endothelial cells of the AGM. Analyzing AGM and peripheral blood of FoxC2 null mouse embryos further confirmed that FoxC2 is a critical factor in the definitive hematopoietic switch. These data establish a pathway that links Notch signaling to activation of blood formation from hemogenic endothelium.

T-2351

NOTCH SIGNALING CONTROLS NEURAL CREST LINEAGE DURING THE DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS

Parinya Noisa[1], Carina Lund[1], Kartiek Kanduri[2], Riikka Lund[2], Harri Lähdesmäki[3], Riitta Lahesmaa[2], Timo Tuuri[1], Taneli Raivio[1]

[1]University of Helsinki, Helsinki, Finland, [2]University of Turku and Åbo Akademi University, Turku, Finland, [3]Aalto University, Helsinki, Finland

Neural crest (NC) cells are specified in embryonic ectoderm in the border of neural plate and epiderm. NC cells are capable of differentiating into various somatic cell types, including melanocytes, craniofacial cartilage and bone, smooth muscle and peripheral nerve cells. Generation of human NC cells would provide a system to study mechanisms of human diseases arising from aberrant NC development (*i.e.* neurocristopathies). Notch signaling plays a significant role during neurogenesis; however, its function during NC development is poorly understood. The primary aim of this study was to investigate the roles of Notch signaling during NC differentiation from human embryonic stem cells (hESCs). Here, we report an efficient protocol to induce hESCs toward NC lineage. With marginal inhibition of BMP and activation of Wnt pathways, we obtained differentiating cells that expressed NC specifier genes, including *PAX3*, *SLUG* and *TWIST1*, termed these cells as NC progenitor cells. These cells could be maintained in the same differentiation conditions without losing their differentiation potential. However, blocking of Notch signaling, either by DAPT or shRNA knockdown of Notch ligand, delayed expression of NC specifier genes. Notch inhibitor, together with FGF8, induced migration of NC progenitor cells. In contrast, ectopic expression of activated Notch, known as Notch intracellular domain (NICD), accelerated the onset of NC specifier gene expression. This result was consistent with the finding that NICD could bind to promoters of NC specifier genes such as *DLX5, PAX3, SLUG and TWIST1*. In addition to NC formation, Notch signaling maintained the NC progenitor state. Under optimal conditions, NC progenitor cells could give rise to neural NC derivatives, such as chondrocytes, osteocytes and adipocytes. Noteworthy, peripheral sensory neurons were favorably generated when Notch signaling was abolished from NC progenitor cells. Taken together, our work highlights, for the first time, the important roles of Notch signaling in 1) formation, 2) migration and 3) derivative differentiation of NC. Our ongoing focus is to investigate the role of Notch signaling in abnormal NC development in CHARGE and Kallmann syndrome pathology with patient-specific induced pluripotent stem cells.

T-2352

FORCED EXPRESSION OF SOX17 CONVERTS MOUSE EMBRYONIC STEM CELLS (MESCS) INTO FUNCTIONAL EXTRAEMBRYONIC ENDODERM STEM (XEN) CELLS

Angela C.H. McDonald[1], Steffen Biechele[1], William L. Stanford[2], Janet Rossant[1]

[1]Developmental and Stem Cell Biology, The Hospital for Sick Children, Toronto, ON, Canada, [2]Sprott Centre for Stem Cell Research, Ottawa Hospital Research Institute, Ottawa, ON, Canada

In addition to the embryo proper, the mammalian conceptus generates two extraembryonic tissues, the trophectoderm and the extraembryonic endoderm (ExEn). In particular, the ExEn is important for patterning of the embryo, gives rise to supporting tissues such as the primary yolk sac, and can be maintained in vitro as self-renewing XEN cells. Little is known about the regulatory networks distinguishing XEN cell lines from the extensively characterized mESC. An intriguing regulatory network candidate is the transcription factor Sox17, which is essential for XEN cell derivation and self-renewal. Previous research has shown that forced expression of Sox17 in mESCs can induce ExEn gene expression. However, the ability of Sox17 to convert mESCs to functional XEN cells has not been explored. To address this, we overexpressed Sox17 in mESCs using a doxycycline-inducible system (Sox17-mESCs), and generated cells with cell morphology indistinguishable from embryo-derived XEN cells. Sox17-mESCs rapidly induce genes encoding basement membrane proteins including Col4a1, Col4a2, Lama1 and Lamb1, as well as Gata4, Gata6 and Sox7 ExEn transcriptions. Subsequently, forced Sox17 expression represses Oct4 and Nanog pluripotency genes. Markers of the molecularly similar definitive endoderm, Cxcr4, Cer1 and Dlx5 are not induced. In contrast to gene expression changes, fluorescent activated cell sorting reveals a stepwise loss of pluripotency cell surface proteins

and a subsequent induction of XEN cell surface proteins. Transgene silencing is observed following Sox17 induction, and these XEN-like cells become transgene independent and stable in culture for greater than 30 passages. Additionally, when fluorescently labeled Sox17-XEN cells are injected into host blastocysts and transferred into pseudo pregnant recipients, these cells integrate and proliferate in the parietal endoderm of E6.5, E7.5 and E8.5 embryos. Sox17 chromatin immunoprecipitation in Sox17-XEN cells, combined with quantitative real-time PCR, reveals that Sox17 is bound to the promoter region of genes encoding basement membrane proteins within 24 hours of induction, and XEN transcription factors by 6 days. In combination with gene and protein expression, these findings suggest that Sox17 expression disengages the mESC regulatory network and assembles the XEN regulatory network. Genome-wide RNA expression and Sox17 binding studies will reveal additional XEN regulatory networks members important for ExEn specification and maintenance.

T-2353

INDUCTION OF HUMAN PLURIPOTENT STEM CELLS TO A PRE-NEUROEPITHELIAL STATE TO GENERATE FLOOR PLATE AND ROOF PLATE PROGENITORS.

Mark Denham[1], Kouichi Hasagawi[2], Dong Zhang[3], Shelley Hough[1], Trevelyan Menheniott[3], Jessie Leung[1], Ben Rollo[3], Don Newgreen[3], Martin Pera[1], Mirella Dottori[1]
[1]University of Melbourne, Parkville, Australia, [2]Institute for Integrated Cell-Material Sciences, Kyoto, Japan, [3]Murdoch Childrens Research Institute, Melbourne, Australia

There are numerous published protocols describing neural differentiation of human pluripotent stem cells, most of which involves inhibition of the BMP and/or activin/nodal pathways, to generate early neural progenitors expressing neuroepithelial markers including Pax6, Sox2 and Nestin. Until recently, these Pax6+/Sox2+ progenitors were considered to be the earliest neural progenitor arising from pluripotent stem cells that can generate most lineages of the nervous system given the appropriate signals. However, recent studies from our laboratory and also of others, found that these Pax6+/Sox2+ neuroepithelial cells are still somewhat restricted in their differentiation potential. Our data shows that certain lineages of the nervous system, including midbrain dopaminergic neurons and neural crest-derived peripheral neurons, arise from an earlier progenitor stage we call ‘pre-neuroepithelial’. This ‘pre-neuroepithelial state’, is defined as Oct4-/Pax6-/Otx2+/Sox2+, and is a pivotal point for cell fate that dictates their commitment to differentiate towards floor plate, roof plate or neuroepithelial cells. Importantly, we determined that inhibition of the GSK3β pathway and the activin/nodal pathways in are involved in differentiating human pluripotent stem cells to this early pre-neuroepithelial stage and this may be achieved using small molecules in chemically defined media. Essentially, using our novel differentiation system, our studies define the earliest progenitor stage during neural induction of human stem cells that give rise to major lineages of the central and peripheral nervous system.

T-2354

IDENTIFICATION OF A NOVEL ENHANCER CLUSTER DOWNSTREAM OF THE SOX2 LOCUS ACTIVE IN MOUSE EMBRYONIC STEM CELLS

Harry Y. Zhou, Felicia Collura, Yulia Petrenko, Jennifer A. Mitchell
Cell & Systems Biology, University of Toronto, Toronto, ON, Canada

Sox2 is a gene that encodes the Sry-related HMG box 2 transcription factor, which plays a critical role in maintaining the pluripotent state of embryonic stem (ES) cells and regulating the development of the central nervous system. Until now, transcription of Sox2 in mouse ES cells and neural stem cells is thought to be regulated by two enhancers known as the Sox2 regulatory region 1 and 2. However, we have recently identified a novel putative enhancer cluster located 100-120 kb downstream of Sox2. This putative enhancer cluster is the top candidate predicted by our computational model based on histone modifications and chromatin-associated protein binding. Evidence suggests that this cluster is active only in mouse ES cells and contributes to the cell-type-specific regulation of Sox2 expression. First, the cluster is bound by a large number of ES-specific transcription factors involved in pluripotency

and self-renewal. Second, it is enriched for p300, H3K4me1 and H3K27ac only in mouse ES cells and it loses these enhancer marks as ES cells differentiate in mesendodermal lineage. Thus, the goal of our current work is to determine whether this putative enhancer cluster regulates Sox2 expression in mouse ES cells. Our chromosome conformation capture (3C) experiments have shown that the enhancer cluster interacts with Sox2 promoter in ES cells but not in embryonic day 11.5 brain tissue or mouse embryonic fibroblasts. Further experiments differentiating mouse ES cells into neural stem cells will elucidate the dynamic changes in chromatin folding at the Sox2 locus. Identified p300 bound regions within the enhancer cluster have been cloned into luciferase reporter vectors to test enhancer activity in mouse ES cells. Further investigation into this novel putative enhancer cluster will yield important insights into the regulation of Sox2 in ES cells and structural differences in chromatin organization between ES and neural stem cells.

T-2355

SKELETAL TERATOGENICITY OF THIRDHAND SMOKE

Nicole R.L. Sparks, Ivann K.C. Martinez, Nicole I. zur Nieden
Department of Cell Biology, Neuroscience, College of Natural and Agricultural Sciences, University of California Riverside, Riverside, CA, United States

Over the last few decades, research regarding cigarette smoke has been mainly focused on firsthand (FHS) and secondhand smoke (SHS). Toxicants found in FHS and SHS have been implicated in several tobacco related health risks including cancers, respiratory complications, and cardiac diseases. In addition, FHS and SHS have been shown to affect bone development, which may lead to an increased risk of developing osteogenic diseases, such as osteoporosis. While the molecular mechanisms behind FHS and SHS teratogenicity are still being elucidated by us and others, recent studies have identified novel constituents that are implicated in tobacco related health hazards summarized under the term thirdhand smoke (THS). THS is a toxic mixture derived from nicotine and other combustible ingredients in tobacco that have reacted with common indoor pollutants. THS and its constituents are absorbed into everyday common household items and then released back into the environment through physical contact. Potentially, THS is viewed to be more hazardous than both FHS and SHS due to its longer half life and chemical transformation time that ranges from seconds to months. Moreover, infants and young children are more susceptible to the health-related risks associated with THS because they have increased contact with THS saturated surfaces. This results in various routes of exposure to the putative toxicants, such as inhalation, ingestion, and dermal transfer.
Research studies have described three common THS constituents: 1-(N-methyl-N-nitrosamino)-1-(3-pyridinyl)-4-butanal (NNA), 4-(methylnitrosamino)-1-(3-pyridinyl)-1-butanone (NNK), and N-nitroso nornicotine (NNN). *In vivo* studies have already suggested that these compounds are carcinogenic and pose risks to a person's health, but how these affect bone development is largely unclear.
Here, we propose that THS constituents can potentially alter bone development similar to firsthand and secondhand smoke. Putative adverse effects of the three THS constituents named above were tested using osteogenically induced human embryonic stem cells as an *in vitro* model for bone development. MTT analysis coupled with calcium measurements determined that exposure to NNN and NNK exhibited a concentration-dependent teratogenic effect, while NNA demonstrated a cytotoxic effect. Astonishingly, this is the first finding, to our knowledge, that a THS constituent can perturb bone development. Ultimately, these results require further elucidation of the molecular mechanisms behind these effects.

T-2356

DIFFERENTIATION OF EMBRYONIC STEM CELLS UNDER MODULATED OXYGEN CONDITIONS INCREASE STAGE 4 NKX6.1+ CELLS AND IN VIVO MATURATION TO BETA CELLS

Amanda Dilenno[1], Anna Kokensparger[1], Jennifer Hollister-Lock[2], Susan Bonner-Weir[2], Clark K. Colton[1]

[1]Massachusetts Institute of Technology, Cambridge, MA, United States, [2]Joslin Diabetes Center, Harvard Medical School, Boston, MA, United States

Pluripotent stem cells (PSC) hold promise for cell replacement therapy and investigation of embryonic development. However, efficient differentiation to desired cell types remains a major obstacle. Most PSC differentiation is performed in high, non-physiological O2, but cells during embryonic development are exposed to much lower O2. Here we report a wide-ranging study showing that physiological O2 markedly influences differentiation to insulin-producing cells. We differentiated CyT49 human embryonic stem cells (hESC) under different, well-characterized pO2 environments, controlling cellular oxygen exposure through adhesion culture on highly O2-permeable silicone rubber membranes, using a modification of the 5-stage protocol reported by ViaCyte, Inc (San Diego, CA) (D'Amour 2006 Nature Biotech). Each stage was examined at multiple controlled high and low oxygen levels, and O2 conditions were identified that increased the fraction of the appropriate intermediate cell type by flow cytometry or increased expression of appropriate genetic markers by real-time PCR. The best differentiation was produced by an oxygen-modulated protocol. Differentiation under 5% O2 from hESC to definitive endoderm (stage 1), primitive gut tube (stage 2), and to posterior foregut (stage 3), then under 20% O2 to pancreatic endoderm (stage 4) and insulin-producing cells (stage 5) gave rise to a cell population that was 43% positive for NKX6.1, after stage 4, and was 10% positive for both c-peptide and NKX6.1 after stage 5. In comparison, differentiation of cells at normoxic oxygen (20% O2) gave rise to a population that is 33% positive for NKX6.1 after stage 4 but 3% positive for both c-peptide and NKX6.1 after stage 5. Both normoxic and the modulated oxygen differentiations produced cells that passively secreted c-peptide into the medium but were not glucose responsive. Pancreatic endoderm markers NKX6.1 and PDX1were increased by a factor of two and four respectively for the controlled-hypoxia (5% stage 1-3, 20% stage 4-5) when compared to the normoxic condition (20% stage 1-5). After differentiation to pancreatic endoderm (stage 4) under the modulated oxygen condition or normoxia, 1 million were implanted under the kidney capsule of SCID/beige mice to allow maturation into functional beta cells. Human c-peptide was detected in serum of 2/8 animals containing oxygen-modulated grafts (one at 12 weeks, the other at 20 weeks post implantation) and 0/8 of animals with normoxic grafts 60 min after stimulation with glucose. Grafts from the same 2 mice transplanted with modulated oxygen differentiation cells had cells positively immunostained for insulin and for a cocktail of non-beta cell hormones. Mice from both groups had cells positive for non-beta cell hormones but no insulin. Based on these results, O2 combined with directed differentiation protocols is a potentially straightforward method that could be applied to future hESC therapy protocols for improved differentiation and maturation to beta cells.

T-2357

PROBING THE POTENTIAL ROLES OF GENOMIC IMPRINTING IN ES CELL DIFFERENTIATION

Yulia Shamis, **Xiajun Li**
Department of Developmental & Regenerative Biology, Ichan School of Medicine at Mount Sinai, New York, NY, United States

The KRAB zinc finger protein ZFP57 is a master regulator of genomic imprinting and it is required for the maintenance of genomic DNA methylation imprint at a large subset of imprinted regions in mouse embryos. We have also demonstrated that it plays a similar role in mouse embryonic stem (ES) cells. Since *Zfp57* is highly enriched in undifferentiated ES cells but down-regulated during ES cell differentiation, we wonder if it may be involved in the maintenance or differentiation of ES cells. We have obtained multiple independent *Zfp57*-null ES clones via homologous recombination by sequential targeting of both alleles of *Zfp57*. These *Zfp57*-null ES clones have similar morphology to undifferentiated ES cells, suggesting that ZFP57 is not required for the maintenance of undifferentiated state of ES cells. As an initial attempt to dissect the potential functions of *Zfp57* in controlling differentiation of ES cells, we have differentiated *Zfp57*-null ES cells into cardiac and vascular lineages. Our preliminary results indicate that the differentiation potentials of *Zfp57*-null ES cells into cardiomyocytes and endothelial cells are significantly impaired, in comparison to those of the wild-type parental ES cells. Since ZFP57 maintains genomic imprinting in ES cells and DNA methylation imprint is lost at a large subset of imprinted regions in *Zfp57*-null ES cells, our data suggest that

genomic imprinting is required for proper differentiation of undifferentiated ES cells into cardiac and vascular endothelial lineages, similar to its functions in mouse embryos.

T-2358

HIGHLY EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO MULTIPOTENT DEFINITIVE ENDODERM USING THE SERUM-FREE AND ANIMAL COMPONENT-FREE STEMDIFF™ DEFINITIVE ENDODERM CULTURE SYSTEM

Michael J. Riedel, Stephanie Lam, Terry E. Thomas, Allen C. Eaves, Sharon A. Louis
STEMCELL Technologies Inc., Vancouver, BC, Canada

The formation of definitive endoderm (DE) from human pluripotent stem cells (hPSCs) is a required intermediate step in the development of more specialized cells of endoderm organs. Recent studies show that hPSCs can be differentiated towards cell types of the liver, pancreas, lung, and intestine. Critical in these processes is the initial development of a highly pure population of DE. Current state-of-the-art protocols for DE differentiation often require fetal bovine serum to allow for high efficiency differentiation across multiple hPSC lines; however, serum may contribute to inconsistent performance. The use of patient-specific human induced pluripotent stem cells (hiPSCs) for disease modeling, drug screening, and cell-based therapies will require a protocol for efficient DE differentiation across many hiPSC lines. To meet the need in the field for improved and standardized reagents and protocols for DE differentiation, we developed a fully defined serum-free and animal component-free medium that results in highly efficient DE differentiation across multiple hPSC lines. Furthermore, we demonstrate that the DE formed using this medium retains the ability to be further differentiated to liver, pancreas, and lung cell lineages.
Human embryonic stem cells (H1 and H9) or hiPSCs (A13700, WLS-4D1, WLS-1C, and WLS-1D) were maintained under defined and feeder-free conditions on Matrigel^{TM} in mTeSR^{TM}1 or TeSR2^{TM}. Clump cultures were dissociated using the non-enzymatic Gentle Cell Dissociation Reagent and re-plated as single cells at high density ($2x10^5$ cells/cm^2) in the presence of Y-27632. Following overnight culture, cells were washed once with DMEM/F12 then incubated for 24 hours in the presence of STEMdiff™ Definitive Endoderm basal medium plus Supplements A and B. A further 3 day culture in STEMdiff™ Definitive Endoderm basal medium plus Supplement B yielded highly enriched DE as assessed by flow cytometic analysis of CXCR4 and SOX17 co-expression. Average differentiation efficiencies ranged from 92.6 ± 1.3% to 84.1 ± 2.8% $CXCR4^+SOX17^+$ cells (mean ± SEM; n = 1 to 18 per cell line) in 6 hPSC lines tested. While expression of the pan-endoderm marker FOXA2 remained widespread after DE differentiation, expression of the anterior foregut endoderm (FE) marker SOX2 was low, indicating no biasing towards an anterior fate during DE induction. Importantly, using published protocols, we demonstrated specification of the DE population enriched in our protocol towards pancreatic, hepatic, and respiratory cells, indicating retention of multipotency towards both anterior and posterior FE lineages.
We have developed a fully defined, serum- and animal component-free medium that provides highly efficient differentiation of hPSCs to DE. Cultures differentiated using this new medium demonstrated robust expression of DE markers and maintained the ability to be directed to multiple endoderm lineages. The protocols described here can be reliably used as the starting point for studies aimed at the generation of endoderm cell types including those of the pancreas, liver, and lung.

T-2361

PRE-TREATMENT OF HUMAN EMBRYONIC STEM CELLS WITH SMALL MOLECULES DURING DEFINITIVE ENDODERM INDUCTION

Yaser Tahamtani[1], Mahnaz Azarnia[2], Ali Farrokhi[1], Nasser Aghdami[1], Hossein Baharvand[1]
[1]Royan Institute, Tehran, Iran, Islamic Republic of, [2]Kharazmi University, Tehran, Iran, Islamic Republic of

Definitive endoderm (DE) formation is the most
important stage that all endodermal organs pass through during their

development. So, in vitro production of definitive endoderm is one of the important issues in stem cell related differentiation studies and can help to efficiently produce endoderm derivatives for therapeutic applications. Using a two-stage differentiation strategy, we treated Royan H6 human embryonic stem cells (hESCs) for one day with “priming” small molecules (Stauprimide, NSC-308848, Rapamycin and CHIR) and for next three days with “inductive” small molecules including LY294002, Cymarin and IDE1/2. In the positive control group, hESCs were treated with Wnt3a (25 ng/ml) and activin A (100 ng/ml) for one day and activin A (100 ng/ml) for the next 3 days (W/A100-A100). Gene expression analysis showed that treatment of hESCs with 100 nM Rapa and 50 ng/ml activin A (Rapa-A50) out of 25 combinations of factors gave rise to higher expression of two DE-specific genes, *SOX17* and *FOXA2*. The gene expression results were confirmed at the protein level and also similar results were obtained after treating 3 other pluripotent cell lines (Royan H2/H5 hECSs and human induced pluripotent stem cell 1) with this regimen. To investigate the in vitro competency of Rapa-A50-induced DE cells for further differentiation into endodermal tissues, these cells as well as W/A100-A100-induced DE cells (as positive control) were further differentiated into pancreatic progenitor (PP) cells using five previously described PP differentiation protocols and then into pancreatic endocrine (PE) cells with three selected protocols according to the *PDX1* expression levels. Expression levels of 10 pancreatic developmental genes were evaluated during DE, PP and PE stages. The results showed that the established protocols were not sufficient to enable universal differentiation into PE but Rapa-A50-induced DE was more competent for PP differentiation in a protocol-dependent manner. As well, Rapa-A50-induced DE was competent to differentiate into hepatocyte-like cells (HLCs) as efficiently as W/A100-A100-induced DE. Additionally, transplantation of Rapa-A50- and W/A100-A100-induced DE cells under the testis capsule of athymic nude mice led to the formation of teratomas which were histologically the same as hESCs-derived teratomas. These data indicated that priming hESCs with Rapa, and inducing them by a lower concentration of activin A could led to DE differentiation with the capability to further differentiate into HLCs and PP cells, but not PE cells. These findings can help to design more efficient chemically defined protocols for DE induction of hESCs and lead to better understanding of the different signaling networks involve in DE differentiation of hESCs.

T-2362

ESTABLISHING A LINEAGE TREE FOR MESODERM AND ENDODERM

Dapeng Yang, Ingo Burtscher, Heiko Lickert
Institute of Diabetes and Regeneration Research, munich, Germany

Establishing a lineage tree for mesoderm and endoderm

Dapeng Yang[1,2], Ingo Burtscher[1,2] & Heiko Lickert[1,2]
[1] *Institute of Stem Cell Research,* [2]*Institute of Diabetes and Regeneration Research, Helmholtz Zentrum München, 85764 Neuherberg, Germany*

In the mouse embryo, gastrulation starts and pluripotent Oct4^{+} epiblast cells undergo epithelial-mesenchymal transition (EMT) to ingress into the posterior primitive streak (PS) region to form mesoderm and definitive endoderm (DE), while the remaining epiblast cell will form the ectoderm (Beddington and Robertson, 1999; Tam and

Loebel, 2007). The progenitor cells of the mesoderm and DE are not identified and the early lineage relationships are not clarified. We have previously noticed that mesoderm and DE is specified already in the epiblast, where Brachyury (T)-positive cells mark distinct distal and proximal epiblast regions that were fate mapped to give rise to anterior vs posterior mesendoderm populations. In this study, we have generated a dual knock-in reporter embryonic stem cell (ESC)-line expression T::GFP and Foxa2-tagRFP that enabled us to identify and characterize mesendoderm progenitors (Foxa2+, T+), the mesoderm lineage (T+) and DE lineage (Foxa2+). Using this system, we analyzed the lineage trees by single cell imaging combined with confocal time-lapse analysis in ESC differentiation culture. Additionally, we purified several lineages at several by FACS in combination with the epithelial surface marker CD24 and established the genome-wide mRNA expression profile. Taken together, our *in vitro* ESC differentiation system nicely recapitulates gastrulation *in vivo* and revealed novel cell biological and molecular insights into the formation of the early mesendoderm lineage.

T-2363

TRANSCRIPTION FACTOR MEDIATED PROGRAMMING OF ES CELLS INTO DENDRITIC CELLS

Istvan Szatmari, Emilia Simo, Erika Takacs, Pal Boto
Biochemistry and Molecular Biology, University of Debrecen, Debrecen, Hungary

Dendritic cells (DCs) are professional antigen presenting cells that are specialized to capture, process and present antigens to T cells in order to modulate immune response. The use of DCs to prime responses to tumor antigens provides a promising approach to cancer immunotherapy but clinically relevant responses have frequently been disappointing, partly due to the properties of the DCs obtained from adult progenitors. Embryonic stem (ES) cells have the potential to give rise to any cell types in the human body, raising exciting prospects to generate DCs from this pluripotent cell source. It was already reported that DCs could be generated from ES cells (ES-DC). However ES-derived DCs had an impaired T cell activation capacity suggesting that some signaling pathways or some factors are missing from these cells. To obtain ES-DCs with enhanced immunogenicity we intend to manipulate their development by transcription factor mediated cellular programming. We have selected 15 DC/myeloid-specific transcription factors to study the expression of these genes and probe the effect on ES cell-derived DC progenitors. The transcript level of these fifteen myeloid/DC specific transcription factors was determined from ES- or bone marrow (BM)- derived DCs. Our data revealed that eleven genes out of the 15 showed a similar expression pattern in both type of cells (ES vs BM derived DCs). Interestingly, 3 genes (Spib, Runx3 and Irf4) showed a lower expression in ES derived DCs. Next we probed the effects of these transcription factors on stem cell derived DC progenitors. We have generated inducible mouse ES cell lines in which these genes can be upregulated by doxycycline treatment (Tet-on system). Our preliminary data indicated that the induction of these transcription factors has a detrimental effect on ES cell-derived mesodermal/blood cell progenitors.

T-2364

THE DYNAMIC TRANSITION OF PLURIPOTENT CELLS INTO ENDODERMAL CELLS VIA A NOVEL INTERMEDIATE CELL

Oded Kopper, Nissim Benvenisty
Department of Genetics, The Hebrew University, Jerusalem, Israel

The derivation of human embryonic stem cells (HESCs) has provided an exceptional tool for the study of human embryogenesis. It enabled the isolation of early human germ layer progenitor cells and the characterization of their gene expression. Yet, there are still many unresolved questions regarding the dynamic transition of a pluripotent cell into a differentiated cell. In this work we generated unique HESC clones harboring mCherry and GFP reporter constructs driven by the promoters of OCT4, a pluripotent marker, and SOX17 an endodermal marker, respectively. We used immunofluorescence and time-laps microscopy in order to trace the transition of HESCs into early endoderm progenitor cells, and demonstrated that this transition occurs through an intermediate cell that expresses both OCT4 and SOX17. Moreover, we could demonstrate that unlike endodermal progenitor cells, in which pluripotent transcription factors such as OCT4 and NANOG have already been shut down, this intermediate cell is not

fully committed to endodermal differentiation but can also shut down SOX17 expression and return to its pluripotent state, pending on the culture conditions. Isolation and gene profile analysis of the pluripotent, endodermal and newly identified endodermal intermediate cells, revealed the dynamic nature of gene expression that induces and promotes endodermal differentiation. Thus, we could identified clusters of genes that are rapidly down or up regulated upon pluripotent cell differentiation into the endodermal intermediate cells and clusters of genes that are down or up regulated only upon differentiation into endodermal progenitor cells. Further analysis enabled to identify key pluripotent and endodermal transcription factors that regulate this dynamic gene expression pattern and thus allows a better view on early endodermal differentiation process.

T-2365

MICROENVIRONMENTS FOR HUMAN EMBRYONIC STEM CELL DIFFEREMTIATION.

Janet Zoldan[1], Shulamit Levenberg[2], Daniel Anderson[1], Robert Langer[1]
[1]Koch Institute for Integrative Cancer research, MIT, Cambridge, MA, United States, [2]Biomedical Engineering, Technion, Haifa, Israel

MICROENVIRONMENTS FOR HUMAN EMBRYONIC STEM CELL DIFFEREMTIATION.
Human embryonic stem cells (hESCs) are becoming an important component of approaches for regenerative medicine, especially within the rapidly expanding field of tissue engineering. To unlock the full therapeutic potential of these cells, it is necessary to develop new approaches for their *in-situ* growth and differentiation towards useful specific sub types. Here, I will describe our efforts to achieve this aim by developing three dimensional (3D) extracellular and intracellular stem cell microenvironments designed to deliver biophysical and biochemical cues. First, I will discuss modeling the germ layer formation process by culturing hESCs on scaffolds with stiffness engineered to model that found in specific germ layers. We show that a different stiffness threshold of the scaffolds promoted differentiation to each germ layer, reminiscent of the forces exerted during the gastrulation process. Second, I will describe an extracellular stem cell microenvironment with biochemical signaling mimicking embryonic vascular development and its *in-vivo* application for preventing limb necrosis in an ischemic hind limb mouse model. Finlay I will introduce an intracellular stem cell microenvironment for delivering silencing genes (e.g, short interfering RNAs) known to participate hESCs differentiation. Efficient siRNA silencing of the kinase insert domain receptor gene, prevented hESCs differentiation into the endodermal germ layer and concurrently increased differentiation towards mesoderm germ layer. These approaches highlight how engineering and stem cell biology can be used synergistically to create microenvironments favorable to the development of specific cell types. These environments can serve as platforms for fundamental research in tissue development, disease mechanisms, or drug testing and hold potential for *in-situ* tissue regeneration applications.

T-2366

DELINEATING THE MECHANISMS AND CONSEQUENCES OF HUMAN EMBRYONIC STEM CELL HETEROGENEITY

Tom Allison
University of Sheffield, Sheffield, United Kingdom

Human embryonic stem cells (hESCs) have been heralded as the future for a variety of uses including toxicology, disease modelling and regenerative medicine. However their clinical applications have been delayed due to the lack of understanding of fundamental decision-making with respect to self-renewal and differentiation.
Accumulating evidence suggests that the states in which hESCs exist may be far more dynamic than previously thought, raising the question as to whether these different sub-states can bias the outcome of cell fate decisions[1, 2]. Using single cell Q.PCR we have found a marked difference in the expression patterns of genes associated with differentiation consistent with the existence of hESCs co-expressing markers for pluripotency and for lineage specification. Two scenarios arise. The first is that these cells are in the process of differentiation and are committed to leave the stem cell compartment. The second is that hESCs can remain within the stem cell compartment whilst also expressing lineage markers.

To test these hypotheses, we have made a GATA6-linked GFP reporter line by zinc finger nuclease mediated gene targeting. Using these reporter cells we have found that GATA6 expressing cells do make stem cell colonies in clonogenic assays indicating that they are still within the stem cell compartment. By qPCR we have also found an increase in the expression of other endoderm makers within the GATA6+ stem cell population, but no increase in mesodermal or ectodermal markers, suggesting that they may indeed exhibit an endodermal lineage bias.
Additionally we have identified four novel antibodies, (CC9, CH8, AG10 & AF12)[3] that detect surface antigens showing positive correlations with the endoderm lineage, providing additional tools for investigating the relationship between subsets of stem cells within the stem cell compartment of human ES cells and endoderm fate decision.

T-2368

MODELING EARLY HEART DEVELOPMENT IN DOWN SYNDROME USING SIBLING HESC LINES

Alexis Bosman[1], Audrey Letourneau[2], Rostyslav Kuzyakiv[1], Flavio Ronzoni[1], Outi Hovatta[4], Stylianos Antonorakis[3], **Marisa Jaconi**[1]

[A]Pathology and Immunology, [B]Genetic Medicine and Development, [C]Medical Genetics and Development, [1]Geneva University, Geneva, Switzerland, [2]Medical Science, Karolinska Insitute, Stockholm, Sweden

Human embryonic stem cells (hESC) carrying known diseases provide excellent models for examining the cellular consequences of a disease from the earliest time in development. Due to differing genetic backgrounds, however, hESC lines are known to display intrinsic differences in their differentiation capacities and epigenetic patterns. These genetic differences result in significant differences upon differentiation, thus making it difficult to detect small variations between diseased and control lines.
Using a hESC sibling model of disease provides a more sensitive approach to detecting small variations due to greater genetic similarity. In this study, we have isolated and characterized a trisomy 21 (T21) hESC model of Down syndrome (DS) using sibling hESC lines as controls. As congenital heart defects (CHD) are the leading cause of morbidity DS, we examined the genetic pathways associated with cardiogenesis to ascertain perturbations in development which may lead to CHD. Upon differentiation, T21-hESC show many significant differences in expression of genes associated with both mesodermal and cardiac development, which is particularly evident with genes associated to the secondary heart field (SHF). Additionally, genes of the T-box transcription factor family were found to be significantly over-expressed in T21-hESC. Some of these perturbations also coincide with known causative genes for CHD observed in the general population. Furthermore, we identified at least one gene located on chromosome 21 which may account for some of these perturbations. Therefore, our work shows for the first time, that T21-hESC and their sibling control lines are a useful model facilitating the identification of differentially expressed genes associated with early cardiogenesis, which may underlie the cause of CHD observed in DS.

T-2371

DERIVATION OF TRANSPLANTABLE PHOTORECEPTORS FROM EMBRYONIC STEM CELLS

Sarah Decembrini[1], Ute Koch[3], Alexandre Moulin[2], Freddy Radtke[3], Yvan Arsenijevic[1]

[A]Unit of Gene Therapy & Stem Cell Biology, Jules-Gonin Eye Hospital, [B]Eye Pathology Laboratory, Jules-Gonin Eye Hospital, [1]UNIL University of Lausanne, Lausanne, Switzerland, [2]Institut Suisse de Recherche Expérimentale sur le Cancer, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Retinal degenerative
diseases resulting in the loss of photoreceptors are a major cause of
blindness. Recently, different groups validated the possibility to reactivate
dormant retinal circuits of degenerating retinas using retinal prosthesis, gene
therapy, cell replacement therapy. Photoreceptor replacement therapy may be
feasible since transplanted photoreceptors, collected directly from the

developing or the adult retina, have been shown to restore some visual function in mice affected by retinal degeneration. Because the developing retina is not a suitable source of renewable photoreceptors, we focused on embryonic stem (ES) cells for their capacity to generate retinal progenitors and photoreceptor cells *in vitro*. In this study, we derived a new transgenic ES cell line in which the reporter gene, the *Crx*-GFP transgene, is expressed in both post-mitotic immature and mature photoreceptors, and assessed the extent to which this protocol recapitulates photoreceptor development *in vitro*. Using a modified 3D-retina induction protocol, GFP-positive photoreceptors appeared in a punctuated pattern into the optic-cup like structures between day 12 and 14 and increased in number reaching the peak of birth between day 18 and 20 of culture. Similarly the intensity of the GFP signal and the alignment of photoreceptors increased over time inside the future outer nuclear layer of *in vitro*-generated retinas. In addition we proved that transplantation of ES cells-derived photoreceptors is feasible. No appearance of tumour formation was detected after transplantation of sorted photoreceptor cells. A few, *Crx*-GFP-positive cells show the presence of outer-segments and synapse ribbons. Experiments are still ongoing in order to assess the functionality of the transplanted cells.

T-2372

MANIPULATING MOUSE EMBRYONIC STEM CELL ELECTROPHYSIOLOGY BY OPTOGENETICS

Feng Tian, Brandi Davis-Dusenbery, Seun Akeju, Nicholas Atwater, Kathryn Koszka, Kevin Eggan
Harvard University, Cambridge, MA, United States

Embryonic stem cell (ESC) is potentially useful in regenerative clinics given its ability of differentiation. Many in vivo directed ESC differentiation protocols have been developed to generate terminally differentiated cells from all the three germ layers. The molecular circuits by which ESC differentiates into each germ layer are also studied. However, less is understood on the role of electrophysiological changes during ESC spontaneous and directed differentiation due to a lack of non-invasive tools to manipulate and visualize ESC electrophysiology. We are particularly interested in asking the possibility whether the neuronal differentiation of ESC will be influenced by depolarization, given the unique expression patterns of Na^+, K^+ and Ca^{2+} channels in pluripotent ESC, neural progenitors and mature neurons. Here we applied the optogenetic tools to manipulate and potentially visualize the electrophysiology of ESC by constructing a channelrhodopsin (ChR) expressing ESC line. Patch clamp and immunohistochemistry experiments convincingly suggest that the ChR-expressing ESC is optically controllable and can differentiate into various types of neurons. By even depolarizing for a short period of time (12 hours) followed by recovery, ChR-expressing ESC shows a dramatic upregulation of neural progenitor markers and a slight downregulation of ESC markers. At the same time, endoderm and mesoderm markers are specifically blocked if depolarizing the ChR-expressing ESC. Long-term influence of optogenetic stimulation on ESC has been observed under both spontaneous and directed differentiation conditions, suggesting after depolarizing ESC facilitates neuronal differentiation. We also showed that directed differentiation of ESC to specific types of neurons such as motor neuron (MN) has also been improved by

optogenetic stimulation, while no MN selectivity of optogenetically enhanced ESC differentiation was found. These changes have not been significantly observed in ESC line not expressing ChR87 under optogenetic stimulation, suggesting that it is the change of electrophysiology but not the background effect of light illumination that promote ESC neuronal differentiation. Further genetics analysis shows that optogenetic stimulation may act through retinoic acid (RA) pathway.

T-2373

INDUCTION EMBRYONIC STEM CELL DERIVED HEPATIC LIKE CELL

Yoshiya Kanoh[1], Fengming Yue[2], Daihachiro Tomostune[2], Hinako Ichikawa[2], Yuto Takahashi[2], Susumu Yoshie[2], Sakiko Shirasawa[3], Tadayuki Yokoyama[3], Ko Okumura[1], Kastunori Sasaki[2]
[1]juntendo university, Tokyo, Japan, [2]shinshu university, Nagano, Japan, [3]Bourbon Corporation, Nagano, Japan

Embryonic stem cell is expected to be applied for regenerative therapy, because they have ability of infinite proliferation and pluripotency. Surely many methods have been proposed to differentiate hepatic cells from embryonic stem cells, but most of them have low efficiency for differentiation, expensive protocols using growth factors and are far from safety in the case of cell processing using virus transfection To solve these problems, we try to establish the new original method using low molecule and to differentiate hepatocytes from embryonic stem cell. The phenotypic changes of ES cell-derived hepatic cells were analyzed with gene analysis, immunostaing analysis in addition to morphologic and functional analyses. These results showed superiority of this method in simplicity,efficiency and economy, which guarantees to create one possible source of hepatic cell for regenerative medicine.

T-2374

ROLE OF PINX1 IN MAINTAINING THE CHARACTERISTICS OF MOUSE EMBRYONIC STEM CELLS

Yuen Ting Lau[1], Chun Kit Li[1], Mary Mui-Yee Waye[2], **Suk Ying Tsang**[1]
[A]School of Life Sciences, [B]School of Biomedical Sciences, [1]The Chinese University of Hong Kong (CUHK), Shatin, N.T., Hong Kong, Hong Kong

Telomerase is crucial for maintaining telomere's length. Its expression/activity is particularly detected in 'immortalized cells' such as cancer cells and embryonic stem cells (ESCs) whereas only little or none is found in somatic cells. It has been suggested that the high level of telomerase activity participates in maintaining the self-renewal property of ESCs by stabilizing the telomere length.
The regulation of telomerase activity is mainly on telomerase reverse transcriptase (TERT), the catalytic subunit of telomerase. Previous studies showed that Pin2/TRF1-interacting protein (PinX1) can directly interact with TERT and regulate telomerase's activity in other cell types. We have recently showed that mouse (m) PinX1 (mPinX1) is present in mESCs. We firstly characterized mPinX1 in terms of its expression levels and sub-cellular localization in undifferentiated mESCs. By qPCR, the expression level of mPinX1 was found to be higher in undifferentiated mESCs than their differentiation derivatives. This hints that mPinX1 may be involved in the maintenance of the undifferentiated state or differentiation processes of mESCs. By confocal microscopy, mPinX1 was found to mainly localize in the nucleolus in undifferentiated mESCs. We also confirmed the

interaction of mPinX1 with mTERT by co-immunoprecipitation.
MESC lines with mPinX1 stably
knocked down or with mPinX1 stably overexpressed were created. Both cell lines
did not show significant changes in the proliferation rate in undifferentiated
state. Interestingly, when the stable cell lines were
subjected to differentiation, both cell lines displayed a decrease in cardiac differentiation
potential. These results hinted a potential role of
mPinX1 in
determining the differentiation potential of mESCs.
Further studies are needed to dissect the molecular mechanisms underlying the observed effects.

T-2375

BETA CATENIN FUNCTIONS PLEIOTROPICALLY IN DIFFERENTIATION AND TUMORIGENESIS IN MOUSE EMBRYO-DERIVED STEM CELLS

Hidenori Akutsu[1], Tohru Sugawara[1], Youki Takezawa[1], Tomoyuki Kawasaki[1], Noriko Okumura[2], Takumi Miura[1], Kenji Miyado[1], Akihiro Umezawa[1]

[1]Dept of Reproductive Biology, Center for Regenerative Medicine, National Research Institute for Child Health and Development, Tokyo, Japan, [2]Dept of Obstetrics and Gynecology, Keio University School of Medicine, Tokyo, Japan

The canonical Wnt/β-catenin signaling pathway plays a crucial role in the maintenance of the balance between proliferation and differentiation throughout embryogenesis and tissue homeostasis. β-Catenin is an essential component of the Wnt/β-catenin signaling pathway and plays an important role in the maintenance of various types of stem cells including adult stem cells and cancer stem cells. However, it is unclear if β-catenin is required for the derivation of mouse embryo-derived stem cells. Here, we have established β-catenin-deficient (β-cat-/-) mouse embryo-derived stem cells (β-cat-/- mESCs) from zygotes without beta-catenin through the fertilization of beta-catenin null sperm and oocyte. The β-cat-/- mESCs were similar to wild-type mESCs in morphology, cellular growth and expression pattern of pluripotency markers. β-catenin is not essential for acquiring self-renewal potential in the derivation of mESCs. However, in the teratoma assay, tumors formed from embryo-derived β-cat-/- ESCs were immature germ cell tumors without multilineage differentiated cell types. All tumors generated from independent β-cat-/- mESC lines showed severe differentiation defects and a grossly undifferentiated cancer-like appearance. Multiple gene expression analysis of wild-type mESCs-derived teratoma, β-cat-/- mESCs-derived tumor and F9 embryonal carcinoma (EC) cells by qRT-PCR using a TaqMan Array revealed that teratomas derived from both mESCs lines were clearly separated from tumors derived from β-cat t-/- mESCs and F9 EC cells. Careful observation of numerous tumor sections of different β-cat-/- mESC lines generated independently in separate animals revealed pathological carcinomatous features resembling heterogeneous mixtures of germ cell tumors (GCTs) composed of seminoma, embryonal carcinoma, and choriocarcinoma characters. To classify GCTs, immunomarkers are vital. Pluripotent stem cell markers such as OCT3/4, NANOG, and SALL4 are used in clinical trials as they are very sensitive and specific markers. In our immunohistochemical analysis, seminoma, embryonal carcinoma and choriocarcinoma of the GCTs generated from β-cat-/- mESCs showed similar staining pattern with human testicular mixed germ cell tumors. We confirmed our findings by qRT-PCR for pluripotent marker. Re-expression of functional β-catenin eliminated their neoplastic, transformed phenotype and restored pluripotency, thereby rescuing the mutant ESCs. Our findings demonstrate that β-catenin has pleiotropic effects in ESCs; it is required for the differentiation of ESCs and prevents them from acquiring tumorigenic character. These results highlight β-cat-/- mESCs are novel research tools for the study of tumorigenesis of testicular germ cell tumors and the function of β-catenin in cell-fate switching between tissue homeostasis and oncogenesis.

T-2376

IDENTIFYING NOVEL MODULATORS OF BLOOD VESSEL FORMATION USING EMBRYONIC STEM CELL DIFFERENTIATION ASSAYS.

Lamis Hammoud, Jodi Garner, Janet Rossant
Program in Developmental & Stem Cell Biology, Hospital for Sick Children, Toronto, ON, Canada

Vascular endothelial growth factor (VEGF), acting through the VEGFR2 (Flk1) receptor, is crucial for blood vessel formation and development. Many events that occur during embryonic vascular development are recapitulated during adult neoangiogenesis, which is critical to tumour growth and metastasis. While the latest antiangiogenic drugs, such as Avastin® (bevacizumab which acts as an anti-VEGF), have been shown to prolong life expectancy in cancer patients, they have serious side effects. Furthermore, relapses often occur, necessitating the need for novel therapeutic targets. The aim of this study was to develop a robust *in vitro* embryonic vascular differentiation assay suitable for small molecule screens to identify novel modulators of angiogenesis. Briefly, mouse embryonic stem (ES) cells containing a vascular reporter (Flk1-EGFP) were aggregated to form embryoid bodies (EBs) by the hanging drop method. These were then grown in a collagen type I gel in the presence of medium supplemented with 10% fetal bovine serum (FBS) and containing one or more of four previously established angiogenic growth factors (VEGF, bFGF, IL-6 and EPO). Flk1 positive sprouts were assessed and quantified after 6-8 days of culture. VEGF-treated EBs showed reproducible Flk1 positive vascular sprouting regardless of the presence or absence of other factors. Using the Cellomics ArrayScan platform, we developed an algorithm, based on the Neuronal Profiling software, to quantify total expression of Flk1 as well as the number of fluorescent sprouts. We then used this algorithm to demonstrate that the assay can detect both positive and negative responses to signaling pathway inhibitors. Treating cultures with a gamma secretase inhibitor (L685458) induced excessive angiogenic sprouting, as expected for a Notch pathway inhibitor and a Flk1 inhibitor (SU5416) blocked sprouting, as predicted
Currently, we are using this vascular differentiation assay to screen a kinome small molecule inhibitor library provided by the Medicinal Chemistry Platform of the Ontario Institute for Cancer Research (OICR). Promising candidates showing quantitative deviation from control cultures have been observed and will be validated in secondary assays. We are also exploring the development of a similar assay in human embryonic stem cells.

T-2377

PROTEOMICS BASED ANALYSIS OF INTERACTION BETWEEN MURINE STROMAL CELLS AND HUMAN EMBRYONIC STEM (HES) CELLS DURING THE HEMATOPOIETIC DIFFERENTIATION.

Lilian Figueiredo Moreira[1], Everton de Brito Oliveira Costa[1], Tathiane Maistro Malta Pereira[1], Maristela Delgado Orellana[1], Elisa Maria de Sousa Russo[2], Dimas Tadeu Covas[1], Virginia Picanco-Castro[1]
[1]Blood Center of Ribeirão Preto-USP, Ribeirao Preto, Brazil, [2]Departament of Clinical Toxicological and Bromatological Sciences, University of São Paulo, Farmaceutical Sciencies at Ribeirão Preto, Ribeirao Preto, Brazil

Mammalian cells proliferate, differentiate, survive and eventually die in the microenvironment called niche, which is formed by cells, extracellular matrix and soluble factors. The dynamic interaction of the cell with its niche has a direct impact on cellular phenotype and gene expression and this contact is an important key regulator of cell during the differentiation process. In the bone marrow, stromal cells support hematopoiesis by producing growth factors, adhesion molecules and matrix proteins. These factors are responsible for homing, growth, survival and differentiation of hematopoietic stem cells (HSCs). Some factors secreted by stromal cells such as IL-3, IL-6, ligands of receptor tyrosine kinase such as stem cell factor (SCF) and Flt-3 ligand, Notch ligands, bone morphogenetic protein 4 (BMP4), erythropoietin (EPO), thrombopoietin (TPO), vascular endothelial growth factor (VEGF) and others are essentials for *in vitro* hES cells hematopoietic differentiation. However if all these know factors are supplied in the culture of ES cells, hematopoietic differentiation cannot be maintained *in vitro* without stromal cells. Direct contact between stromal cells and HSCs is also crucial for keeping HSCs in a primitive state. These findings suggest that supportive cells in the bone marrow express unidentified molecules that regulate hematopoiesis. Here we have used OP9 mouse stromal cells to promote the hES cells differentiation into all lineages of hematopoietic cell, because this stromal cell line express proteins necessary for regulating hematopoiesis. The H1 hES cell line was co-cultured with OP9-GFP cells for nine days in α-MEM medium with 20% FBS. After this time, we observed the pick of differentiation into hematopoietic progenitors cells. We obtained from $1x10^6$ H1 cells after co-culture with OP9-GFP

cells a population positive for CD34 (25,9%), CD43 (40,1%), CD45 (4,6%), CD31 (18,4%). We used proteomic analysis to identify molecules that can induce and/or maintain hematopoietic differentiation *in vitro*. OP9-GFP cells were analyzed in two situations: before the co-culture and nine days after co-culture with H1 cells. The OP9-GFP cells were separated from H1 cells by flow cytometry. Proteins identified by LC MS/MS analysis of nuclear, cytoplasmic and membrane subfractions of OP9-GFP were prepared and analyzed in a mass spectrometer LTQ-Orbitrap type (Thermo-Finnigan). The data were automatically processed by Computational Proteomics Analysis System-CPAS. In our first analysis was detected a total of 762 proteins. Among them, 370 proteins were found only in OP9-GFP before co-culture with ES cells, 109 proteins were exclusively found in OP9-GFP after co-culture and 283 proteins were found in both situations. Hundred eight four proteins were up-regulated in OP9-GFP cells after co-culture with H1 ES cells and the most of them are associated with cell death and survival, hematological system and tissue morphology. In the 25 highest-ranking proteins found in the co-culture, some important proteins involved in hematopoietic process such as Hspa9 and PICALM were found. With further validation, these proteins may provide a useful tool for identifying genes involved in the hematopoietic differentiation process and the handling thereof may provide a more efficient differentiation protocol.
Financial supported: CNPq, CAPES and FAPESP

T-2378

ACTIVIN-CONJUGATED ELECTROSPUN NANOSTRUCTURES FOR ENDODERMAL DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

Mohammad Kazemi-Ashtiani[1], Mojgan Zandi[1], Jalal Barzin[1], Yaser Tahamtani[2], Hossein Ghanian[1], Hossein Nezari[2], Hossein Baharvand[2]

[1]Biomaterials, Iran Polymer and Petrochemical Institute, Tehran, Iran, Islamic Republic of, [2]Stem Cells and Developmental Biology, Royan Institute, Tehran, Iran, Islamic Republic of

Human pluripotent stem cells (hPSCs) can be induced to differentiate into three primary germ layers, ectoderm, mesoderm and endoderm. Mass production of functional somatic cells by in-vitro endodermal differentiation of hPSCs is considerable for curing of diseases related to endoderm-derived organs such as liver and pancreas. Present protocols for endoderm differentiation are limited to optimization of soluble factors (e.g. growth factors) as their concentration, composition and treating time. In this work, Activin conjugated polycaprolactone nanofibers were used for hPSCs culture in order to mimicking matrix-bound signaling of Activin. Immobilization of Activin increased endodermal markers while physically absorbed Activin showed low expression of these marker genes. Our results demonstrate that covalently bound Activin retains its ability to stimulate differentiation of hPSCs by activating specific signaling pathways in a manner consistent with the biochemical activities of Activin reported previously. These results suggest that it should be possible to develop chemically stable and completely synthetic substrate containing recombinant growth factors and biocompatible polymer for use in a broad range of application including cell culture, in vitro stem cell differentiation, cell-based toxicological screens and regenerative medicine.

T-2381

STAGE SPECIFIC INTEGRATION OF β-CAT AND SMAD SIGNALLING PATHWAYS DURING EARLY HESC FATE SPECIFICATION TOWARDS ENDODERM

Nina S. Funa[1], Karen Schachter[2], Katja Hess[1], Jenny Ekberg[1], Qianren Jin[1], Henrik Semb[3]

[1]Stem Cell and Pancr Dev Biology, Lund University, Lund, Sweden, [2]DanStem, Copenhagen, Denmark, [3]DanStem, Copenhagen, Sweden

Induction of definitive endoderm (DE) is the first critical step towards formation of differentiated cell types from endodermally derived tissues such as insulin producing beta cells of the pancreas. DE formation in the embryo passes through the intermediate step of primitive streak (PS) formation, which has the potential to form either mesoderm or DE. Knowledge remains limited on the mechanisms that regulate hESC DE formation in existing in vitro proto-

cols, and to what extent they mimic differentiation in the mouse embryo. Numerous studies have described Nodal/Activin signalling cues as the regulatory determinants that trigger the switch between pluripotency and early endoderm differentiation. Nodal expression is induced and maintained by Wnt signalling, and mouse embryos lacking either Nodal or β-catenin fail to form the primitive streak. Most hESC DE protocols to date rely on addition of high concentrations of Activin A (AA), with or without Wnt3a in further attempts to mimic in vivo development.
In our lab, we have tried to enhance protocol efficiency and stability by dissecting the spatiotemporal concert of early events during which the cells are specified towards endoderm. Gsk3β regulates β-catenin degradation and Gsk3β inhibitors are commonly used as Wnt signalling surrogates. We show that Gsk3β inhibition by CHIR99021 prior to AA treatment effectively induces conversion of hESCs to PS (T, MIXL1, EOMES, GSC) through β-catenin mediated NODAL transcriptional activity and Smad2/3 phosphorylation. Gene targeting through β-cat/Smad2 siRNA knockdown and ALK4/7 inhibition by SB432152 during CHIR treatment inhibits PS and further progression towards DE (FOXA2, SOX17, CXCR4, CER) despite subsequent addition of AA. CHIR treatment induces NODAL in a temporal manner, which then decreases the requirement for addition of exogenous AA. This leads to a higher degree of homogeneity, compared to the conventional induction protocols for obtaining DE.

T-2382

IN VITRO DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO INSULIN-SECRETING CELLS

Youngjin Kim, Yong-Mahn Han
KAIST, Daejeon, Korea, Republic of

Insulin-secreting cells will be an alternative cell source for curing diabetes. Several approaches have been tried to produce the insulin-secreting cells from human pluripotent stem cells such as ESCs and iPSCs. Here, we represent an efficient protocol for differentiating hESCs into insulin-producing cells. Combined activation of Activin A/Nodal and Wnt signalings induced differentiation of hESCs into definitive endoderm (DE). SOX17 and FOXA2 - double expressing DE cells were differentiated into pancreatic endoderm by inhibition of TGF-β and Shh signalings. Then, PDX1-expressing cells were treated with inhibitor of Notch signaling for endocrine commitment. Activation of cAMP signling enhanced insulin secretion in a glucose-dependent manner. The insulin-positive cells also expressed β-cell specific transcription factors. Also, insulin-expressing cells could be produced from hiPSCs by this stepwise protocol. Our results demonstrate that insulin-secreting cells could be efficiently derived from hPSCs by step-wise modulations of signaling pathways.

T-2383

DISTICT TRANSCRIPTIONAL PROFILES OF ANGIOBLASTS DERIVED FROM HUMAN EMBRYONIC STEM CELLS

Wonhee Suh, Sun-Hwa Song, Koung=Li Kim
AJOU Univ, Suwon, Korea, Republic of

Identification of differentially expressed genes in angioblasts derived from human embryonic stem cells (hESCs) is of great interest for elucidating the molecular mechanisms underlying human vasculogenesis. The aim of this study was to define hESC-derived angioblasts at the clonal level and to perform comparative transcriptional analysis to characterize their distinct gene expression profiles. In a clonal analysis performed in cell-specific differentiation media, hESC-derived $CD34^+CD31^+$ cells were identified as angioblasts in that they exhibited a significantly higher ability to form endothelial cell (EC) and smooth muscle cell (SMC) colonies than $CD34^+CD31^-$ and $CD34^-$cell populations did. Microarray analysis showed that many genes involved in vascular development and signaling transduction were overexpressed in hESC-derived $CD34^+CD31^+$ cells, whereas those related to mitosis, the DNA damage response, and translation were substantially downregulated. In addition, comparative gene expression profiling of hESC-derived $CD34^+CD31^+$ cells and human somatic primary vascular cells demonstrated that hESC-derived $CD34^+CD31^+$ cells expressed key genes involved in the EC and SMC differentiation processes, which supports the result that hESC-derived $CD34^+CD31^+$ cells are bipotent angioblasts. Our results may provide insights into the iden-

tity and function of hESC-derived angioblasts and may also facilitate further investigation of the molecular mechanisms regulating human embryonic vasculogenesis.

T-2384

KEY UPSTREAM REGULATORS OF WNT SIGNALING DURING MIDBRAIN DOPAMINE DIFFERENTIATION IN HES CELLS

Jingli Cai, Stephanie Schleidt, Joshua Pelta-Heller, Danielle Hutchings, Gregory Cannarsa, **Lorraine M. Iacovitti**
Dept. of Neuroscience, Thomas Jefferson University, Philadelphia, PA, United States

Although small molecule inhibitors of the BMP (Dorsomorphin/DM) and TGF-ß (SB431542/SB) signaling pathways were commonly used in protocols to generate midbrain dopamine (mDA) neurons from human embryonic stem (hES) cells, the role that these substances played in the mDA differentiation process had not been studied. We report here that simultaneous treatment of early stage stem cell cultures with DM and SB greatly increases the relative number of cells positive for the DA specifying marker Lmx1a and DA neuronal marker TH. The transient inhibition of constitutive BMP signaling, either alone or in combination with TGF-ß inhibition through pSMAD1, 5, 8 and pSMADs2, 3, is critically important in the upstream regulation of Wnt1-Lmx1a signaling in mDA progenitors. We postulate that the mechanism via which DM or DM/SB mediates these effects involves the up-regulation in SMAD interacting protein 1 (SIP1), which results in greater repression of the Wnt antagonist, secreted frizzled related protein 1 (Sfrp1) in stem cells. Accordingly, knockdown of SIP1 reverses the inductive effects of DM/SB on mDA differentiation while Sfrp1 knockdown/inhibition mimics DM/SB. The rise in Wnt1-Lmx1a levels in SMAD inhibited cultures is, however, accompanied by a reciprocal down-regulation in SHH-Foxa2 levels leading to the generation of very few TH+ neurons that co-express the critical midbrain floor plate factor, Foxa2. If however, exogenous SHH/FGF8 is added along with SMAD inhibitors, equilibrium in these two important pathways is achieved such that authentic (Lmx1a+Foxa2+TH+) mDA neuron differentiation is promoted while alternate cell fates are suppressed in stem cell cultures. These data indicate that activators/inhibitors of BMP and TGF-ß signaling play a critical upstream regulatory role in the mDA differentiation process in hES cells.

T-2385

TRANSLOCATED EMBRYONIC STEM CELLS AS AN IN-VITRO MODEL FOR STUDYING HUMAN IMPLANTATION FAILURE

Michael Telias[1], Yael Kalma[1], Tsvia Frumkin[1], Ami Amit[2], Dalit Ben-Yosef[1]
[A]Mayer Wolfe PGD - Embryonic Stem Cell Laboratory, [B]Racine IVF Unit, [1]Tel Aviv Sourasky Medical Center (Ichilov), Tel Aviv, Israel

Objectives: Implantation is a complex process that requires a functionally blastocyst and a receptive endometrium, and begins with attachment of the trophectoderm to the endometrium. The trophectoderm cells will then differentiate into trophoblast lineages. Implantation failure is one of the main causes of infertility, and can results from both maternal factors and abnormalities in the preimplantation embryo. Derivation of human embryonic stem cells (hESCs) and their ability to differentiate into trophoblasts have significantly improved our knowledge regarding the early events in trophoblast differentiation in humans.
The aim of this work is to study trophoblast differentiation and the mechanism underlying early stages of implantation using a hESC line carrying the unbalanced translocation t(11;22).
METHODS: Fluorescent in-situ hybridization and karyotype analysis were performed to analyze t(11;22). The hESC line was characterized by RT-PCR and FACS analysis for pluripotent markers. Differentiation potential was assessed by spontaneous differentiation into teratomas, as well as by in-vitro differentiation into trophoblasts. Trophoblast development was assessed by measuring βhCG secretion, βhCG immunostaining and by gene expression of trophoblastic markers.
RESULTS: We derived the first hESC line carrying an unbalanced t(11;22) which we labeled Lis05_t(11;22). This hESC line showed the typical morphological and molecular characteristics of a hESC line. It was functionally pluripotent,

giving rise to derivatives of all three germ layers. Lis05_t(11;22) hESCs specifically failed to differentiate into trophoblasts, as evidenced by their failure to secrete βhCG. Gene expression analysis demonstrated inactivation of CDX2. **CONCLUSIONS:** Lis05_t(11;22) is presented as a valuable human in-vitro model for studying the mechanisms underlying implantation failure. We expect that by comparing our hESC lines that have different translocations to control hESCs we will be able to highlight the molecular mechanism underlying early stages of trophoblast differentiation and embryo implantation.

T-2386

DIRECTED DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO FUNCTIONAL OVARIAN GRANULOSA-LIKE CELLS

Chen-wei Lan[1], Hsin-Fu Chen[2], Hong-Nerng Ho[3]
[1]Institute of clinical medicine, National Taiwan University, Taipei, Taiwan, [A]Graduate Institute of Medical Genomics and Proteomics, Department of Obstetrics and Gynecology, [B]Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, [2]National Taiwan University Hospital and College of Medicine, Taipei, Taiwan

Granulosa cells are playing an important role for the development and maturation of oocytes. Dysfunction of granulosa cells may lead to abnormal folliculogenesis and subfertility, such as polycystic ovary syndrome (PCOS), and premature ovarian failure/insufficiency (POF/POI). Recent studies have showed that human pluripotent stem cells have an unlimited capacity for self-renewal and can differentiate into all the
specialized cells. This study aimed to establish a culture system through multi-step approaches comprising *in vitro* treatments with cocktails of growth factors to direct the differentiation of human embryonic stem
cells and induced pluripotent stem cells (iPS) from patients with POF into
granulosa cells. Gene expression analyses showed the progress of human pluripotent stem cells to primitive streak-mesendoderm, intermediate plate mesoderm, and finally to functional granulosa-like cells that expressed the granulosa cell-specific forkhead transcription factor *FOXL2*, estrogen synthetase *CYP19A1*, anti-Müllerian hormone (*AMH*), the type 2 AMH receptor (*AMHR2*), and the follicle stimulating hormone receptor (*FSHR*). Protein expression analyses showed that surface markers AMHR2 and FSHR and steroidogenic enzyme CYP19A1 levels in differentiated human pluripotent stem cells were significantly higher than in undifferentiated cells. These granulosa-like cells were also capable of aromatizing testosterone to estradiol and producing AMH, suggesting that they were biologically functional. In conclusion, we successfully established a protocol to generate functional ovarian granulosa-like cells from human pluripotent stem cells. The derivation of these cells will become an important tool for the study of the mechanisms underlying ovarian steroidogenesis and potential application of these cells in human reproduction.

T-2387

VULNERABLE EPIGENETIC STATUS OF IMPRINTED GENES DURING IN VITRO DIFFERENTIATION OF HESCS INTO SPECIALIZED CELL TYPES.

Sang-Wook Park, Jihoon Kim, Yong-Mahn Han
KAIST, Daejeon, Korea, Republic of

Genomic imprinting is stably maintained during mammalian development. However, little is known regarding the epigenetic dynamics of imprinted genes during human development. Here, we show dynamic epigenetic changes in imprinted genes in human embryonic stem cells (hESCs) during in vitro differentiation into specialized cell types. Out of 10 imprinted genes with single nucleotide polymorphisms, mono-allelic expression for H19, KCNQ1OT1, and IPW, and bi- or partial-allelic expression for OSBPL5, PPP1R9A, and RTL1 were stably retained in H9 hESCs throughout in vitro differentiation, representing imprinting stability. However, three imprinted genes (KCNK9, ATP10A, and RASGRF1) exhibited modifications to allelic status in a lineage-specific manner during in vitro differ-

entiation. In particular, imprinted genes (SLC22A2, SLC22A3, and IGF2R) in IGF2R imprinted cluster showed partial-gain-of imprinting in various hESC-derivatives. Differential methylation at differentially methylated regions (DMRs) of some imprinted genes was hypermethylated in a lineage-specific manner during in vitro differentiation of hESCs. These findings suggest that the epigenetic status of imprinted genes may be vulnerable in a lineage-specific manner in hESCs during in vitro differentiation.
This research was supported by the NRF Stem Cell Program (2011-0019509) funded by MEST, Republic of Korea.

T-2388

A ROLE FOR MECHANICS IN EMBRYONIC STEM CELL DIFFERENTIATION

Kathryn A. Rosowski, Aaron F. Mertz, Eric Dufresne, Valerie Horsley
Yale University, New Haven, CT, United States

In order for embryonic stem cells (ESCs) to differentiate into all different cell types of the body, they must lose their pluripotency and be specified to the correct cell fate in a timely and ordered fashion. Although the changes in gene expression through transcriptional and epigenetic regulation are well studied, less is known about the mechanical signals influencing this process. Mechanical cues likely play a significant role in ESC differentiation, as previous studies have shown that cues from the physical environment influence multipotency and cell fate decisions in many types of stem cells. We are studying the mechanical cues that influence the pluripotency and differentiation of human ESCs (hESCs). Utilizing traction force microscopy, we have measured cellular forces in hESC colonies. We find the strength of interaction between cells and their substrate varies within a colony. These different interactions correlate with distinctive cytoskeletal organization and differential loss of pluripotency. Based on these data, we hope to modulate mechanical and physical aspects of culture conditions to develop differentiation schemes that increase the efficiency of differentiation. Additionally, these findings shed light on how physical mechanisms influence pluripotency and may aid in the clinical use of hESCs for cell therapies.

T-2391

MIR-200C DIRECT TARGET GATA4 AND REGULATE HUMAN EMBRYONIC STEM CELL RENEWAL AND DIFFERENTIATION

Hsiao-Ning Huang[1], Shao-Yin Chen[1], Shiaw-Min Hwang[2], Nianhan Ma[3], Frank Leigh Lu[4], **Jean Lu**[1]
[1]Academia Sinica, Taipei, Taiwan, [2]Food Industry Research and Development Institute, Hsinchu, Taiwan, [3]National Central University, Taoyuan, Taiwan, [4]National Taiwan University Hospital and National Taiwan University, Taipei, Taiwan

Human embryonic stem cells (hESCs) are unique in its self-renewal and pluriopotency ability. hESCs can differentiate into embryoid bodies (EBs) that includes ectoderm, mesoderm, and endoderm which mimics the embryonic development process. However, the molecules required for hESC renewal and EB formation are not fully revealed yet. miR-200 family is well-known for its roles in blocking epithelial-mesenchymal transition (EMT) transition and block tumor progression. Here we demonstrate the critical roles of miR-200c in hESC renewal and differentiation.
We investigate the expression amounts of miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429), and found miR-200c expression is the most abundant genes expressed in undifferentiated hESCs. miR-200c is significantly downregulated upon EB formation. miR-200c is required for hESC renewal and the expression of multiple stem cell key regulators including Nanog, Sox2, and c-Myc. miR-200c directly target the 3'UTR of primitive endoderm marker GATA binding protein 4 (GATA4). The inhibition of GATA4 partially restored the hESC renewal function in the presence of miR-200c inhibitor. In contrast, overexpression of miR-200c inhibits EB formation and downregulates the differentiation markers of all three lineages. The inhibition of GATA4 also blocks EB formation and the overexpression of GATA4 can partially rescue the EB formation upon miR-200c overexpression. The expression of miR-200c can be partially inhibited by the activin inhibitor, which can explain the downregulation of miR-200c upon hESC differentiation. We found that miR-200c is the critical factors that determine hESC renewal and differentiation, parts of its function is mediated through GATA4.

T-2392

IDENTIFICATION OF AN ADHESION MOLECULE FOR ISOLATION OF HEMATOPOIETIC CELLS AND CARDIAC CELLS FROM PLURIPOTENT STEM CELLS

Katsuhisa Tashiro[1], Nobue Hirata[1], Tomoko Yamaguchi[1], Hiroyuki Mizuguchi[2], Kenji Kawabata[1]

[1]National Inst of Biomedical Innovation, Ibaraki, Osaka, Japan, [2]Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka, Japan

Adhesion molecule X is a component of the tight junction complex. It is also shown that X is expressed in the heart in developing embryo, and the expression level of X is low in hematopoietic cells. In the present study, we found that undifferentiated mouse embryonic stem cells (ESCs) potently expressed X, whereas both the cells expressing high levels of X (X^{high} cells) and the cells expressing low levels of X or lacking the X ($X^{low/-}$ cells) were present in mouse ESC-derived embryoid bodies (EBs) cells. We next investigated whether potentially hematopoietic cells and potentially cardiac cells could be separated from mouse EBs based on X expression. A colony assay after the purification of X^{high} and $X^{low/-}$ cells from EBs revealed that immature hematopoietic cells with the colony forming potential were highly enriched in $X^{low/-}$ cells. Increased expression of the hematopoietic transcription factors, such as *Scl/Tal-1* and *Runx1*, was also observed in $X^{low/-}$ cells. In addition, purified $X^{low/-}$ cells could efficiently proliferate on OP9 stromal cells in the presence of hematopoietic cytokines. On the other hand, the expression levels of cardiac progenitor marker gene, including *Mesp1* and *Gata-4*, in X^{high} cells were higher than those of $X^{low/-}$ cells. X^{high} cells also could differentiate into cTnT-expressing cardiomyocytes on OP9 cells, but almost no cTnT-expressing cells were observed in $X^{low/-}$ cells, suggesting the existence of cardiac progenitor cells in X^{high} cells. Furthermore, we observed the X^{high} cells and $X^{low/-}$ cells in mouse induced pluripotent stem cell (iPSC)-derived EB cells, and showed that cardiac cells and hematopoietic cells could be differentiated from iPS-EB-derived X^{high} cells and $X^{low/-}$ cells, respectively. These results showed that hematopoietic progenitor cells and cardiac progenitor cells in mouse ES-EB and iPS-EBs could be easily isolated based on the expression of X. Therefore, an adhesion molecule X would be a potent cell surface marker for purification of hematopoietic cells and cardiac cells from mouse pluripotent stem cells.

T-2393

DIFFERENTIATION OF OLFACTORY PLACODAL CELLS AND THEIR DERIVATES FROM HUMAN PLURIPOTENT STEM CELLS

Carina S. Lund[1], Parinya Noisa[1], Karolina Lundin[1], Timo Tuuri[2], Taneli Raivio[1]

[1]Physiology, Institution of Biomedicine, Helsinki, Finland, [2]Biomedicum Stem Cell Center, Molecular Neurology Research Program, Helsinki, Finland

Preplacodal ectoderm develops at the anterior neural plate border region and gives rise to the cranial sensory placodes including the olfactory placode (OP), a proposed source of GnRH neurons. During embryonic development, GnRH neurons migrate from the OP to the hypothalamus where they control the function of the reproductive axis. Patients with Kallmann Syndrome (KS) display defective development and/or migration of GnRH neurons, which is clinically manifested as reproductive failure and decreased/absent sense of smell. To understand the developmental mechanisms underlying KS, we have generated a differentiation protocol for OP and its derivates by using human pluripotent stem cells. The differentiation protocol is based on known signaling cascades in the neural plate border region. In human embryonic stem cells (hESCs), the activation of FGF signaling and inhibition of BMP and Wnt pathways induce the expression of several preplacodal and olfactory placodal markers such as EYA2, DLX5, FOXG1 and PAX6 in. When the hESC derived OP-like cells were further differentiated towards GnRH neuron identity a considerable increase in GNRH1 expression was observed. A detailed characterization of the prospective GnRH neurons by qPCR, immunocytochemistry, flow cytometry and electrophysiology is currently on-going. In the

second phase of this project, the differentiation potential of KS patient-derived induced pluripotent stem cells into GnRH neurons and other OP derivates will be evaluated. In conclusion, this project will shed new light on the early specification of the human OP and GnRH neurons, as well as the pathology of KS.

T-2394

ACTIVIN A GENE EXPRESSION IS INFLUENCED BY BMP-4 DURING MOUSE ES CELL OSTEOGENIC DIFFERENTIATION

Rubens LC Tavares, Bruno M. Camargos, Helen L. Del Puerto, Aroldo F. Camargos, Fernando M. Reis
Obstetrics and Gynecology, Federal University of Minas Gerais, Belo Horizonte, Brazil

Objective: To study if the Activin A gene expression is influenced by BMP-4 during in vitro osteogenic diferentiation of mouse embryonic stem cells (ESC).
Methods: This is a prospective study, carried out with a newly isololated mouse ESC which was differentiated into osteogenic precursors. Real time PCR was performed for RUNX2, Bone Alkaline Phosphatase, osteocalcin and activin A receptors. We correlated the expression of Activin A in osteogenic precursors with and without BMP-4 culture.
Results: Gene expression of activin A is expressed in undifferentiated mouse ESC, and gradually increases with time of cell culture, particularly with the use of BMP-4, during in vitro ES cell osteogenec differentiation. The osteogenic differentiation protocol used revealed some gene expression of undifferentiation markers even in the final stage of differentiation, which can be a limiting factor for use in cell therapy.
Conclusions: Activin A may play a role on the undifferentiation state of mouse embryonic stem cells in vitro. Activin A may be important during in vitro osteogenesis of mouse ES cells, and its activity is increased by BMP-4 during cell differentiation into bone precursors.
Study funding: The National Intitutes of Hormones and Women's Health. Federal University of Minas Gerais.
There is no competing interest.

T-2395

HTH-GFP REPORTER STEM CELL LINE FACILITATES PARKINSON'S DISEASE STUDY BY ALLOWING FOR HIGHLY ENRICHED MIDBRAIN DA NEURONS DERIVED FROM HUMAN ES CELLS.

Stephanie Schleidt, Jingli Cai, Joshua Pelta-Heller, Lorraine Iacovitti
Neuroscience, Thomas Jefferson University, Philadelphia, PA, United States

Neurodegenerative diseases will become increasingly prevalent in the coming decades, as the global population ages. Despite significant effort in financial, human, and time resources, the etiology of most of these diseases is unclear and cures remain elusive. Parkinson's disease (PD) is the second leading neurodegenerative disease on the planet. By the time symptoms appear three quarters of the dopamine neurons in the midbrain have irrevocably disappeared and no means exist to halt this continuing decay. Cell replacement therapy remains a promising treatment option. Much progress has been made to identify and utilize processes and pathways governing dopaminergic (DA) differentiation. The application of these findings can ultimately lead to adequate means for cell replacement in the affected areas. We and others have shown that embryonic stem cells or induced pluripotent stem cells can be made to commit to a DA neuron cell fate, expressing the same set of genes as the native cell. However, for reasons unknown as of yet, the cultures remain highly heterogeneous. The resulting heterogeneity renders such a culture impractical as a disease model or for the development of an effective therapy. This situation hampers the field's ability to study PD in the vitro, which has far reaching consequences for the advance of therapeutic approaches. Here we report our protocol to establish a human TH-GFP reporter cell line. Our lab has previously constructed a GFP reporter vector driven by 11kB of the human TH promoter which shows exceptionally faithful expression in the midbrain of transgenic mice. In preliminary studies, we have used the hTH-GFP-polyA fragment from that vector and inserted it into a pZDonor-puro vector. Subsequently, using the commercially available CompoZr® Targeted Integration Kit-AAVS1, which utilizes zinc finger nucleases, we have inserted the hTH-GFP transgene into a safe harbor integration site (AAVS1 on chromosome 19). Our differentiation protocol, followed by fluorescence-activated cell (FAC) sorting enrichment steps, yielded 94% TH+ positive cells, and a simple modification to our previous protocol

increased the number of TH+ cells 5-fold. Currently, no cell surface marker for differentiating dopamine neurons is available that would allow us to select cells by FAC sorting. This TH-GFP cell line enables us to search for such markers in the future as well as investigate established Parkinson's disease models on a purified culture of midbrain DA neurons and identify the environment required for the well-being of these cells in culture. Purified reporter cells can also be used in proof-of-principle transplant studies in animal models of PD. In the near future, we will adapt these procedures to create patient specific reporter iPS cell lines.

T-2396

REGENERATION OF THE FUNCTIONAL PHOTORECEPTORS FROM HUMAN EMBRYONIC STEM CELLS

Myung Soo Cho[1], Dae Hoon Yoo[1], Seul Ae Song[1], Yong-ri Koo[2], Joo-young Shin[2], Jung Hyun Park[3], Hyeong Gon Yu[2]
[1]Jeil Pharmaceutical Co. Ltd., Seoul, Korea, Republic of, [2]Department of Ophthalmology, College of Medicine, Seoul National University, Seoul, Korea, Republic of, [3]Department of Ophthalmology, Seoul Paik Hospital, Inje University, Seoul, Korea, Republic of

Despite numerous attempts to generate photoreceptors from human pleuripotent stem cells, the problem of efficiency and long periods of time required for photoreceptor derivation remain one of the many obstacles in the introduction of these cells in the therapy of retinal degenerative diseases. We will report our simplified and efficient differentiation method into photoreceptors using our previously reported spherical neural mass (SNM) derived from human embryonic stem cells (hESCs).
SNMs were fragmented mechanically and co-cultured with retinal pigment epithelial cells (RPEs) such as ARPE-19 or hESC-derived RPEs, in neural differentiation medium with N2 and B27. SNMs are neural progenitor cell masses derived from hESCs, and are cryo-preservable for extended periods of time. Photoreceptors could be detected within 10-14 days after the co-culture in minimal culture conditions. These cells formed short neurites and expressed markers specific for rod and cone photoreceptor cells such as RHODOPSIN, OPN1SW, and OPN1LM and showed functional improvement by electroretinogram after transplantation into animal models. Generation of photoreceptors from hESC-derived SNM with a simplified and efficient method using RPE co-culture with minimal addition of supplements may faciliate the use of these cells in the therapy of retinal degenerative diseases. These strategies in our study could be helpful for functional recovery after RPE transplantation. These strategies in our study could be helpful for functional recovery after RPE transplantation. This study was supported by grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea(A120465).

T-2397

ASSESSING THE DEVELOPMENTAL POTENTIAL OF PLURIPOTENT STEM CELL LINES DERIVED BY DIRECT REPROGRAMMING AND SCNT.

Valentina Lo Sardo, Jennifer Hazen, Kristin Baldwin
Molecular and Cellular Neuroscience, Scripps Research Institute, La Jolla, CA, United States

Pluripotent stem cells (PSCs) include embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and somatic cell nuclear transfer-derived embryonic stem cells (SCNT-ESCs). The utility of PSCs depends on their capacity to reproducibly give rise to desired cell lineages. However, in practice individual PSC lines often differ dramatically in their propensity to generate different cell lineages. At present, no clear means to identify the pluripotency or lineage bias of particular cell lines has emerged. The most stringent test for pluripotency in mouse cell lines is the tetraploid embryo complementation (TEC) assay in which a line is tested for its ability to give rise to all tissues in an organism. One hypothesis would be that PSC lines which give rise to adult animals in TEC assays would exhibit improved developmental potential when subjected to in vitro differentiation assays. To test this hypothesis we have subjected a set of genetically matched ESCs, iPSCs and SCNT-ESCs to the TEC assay and to in vitro differentiation assays. We observe that all classes of PSCs exhibit significant intra-class variability in producing specific cellular lineages in vitro. In addition we show that developmental potency in vitro is not correlated with the ability of a particular PSC line to produce viable full term mice in TEC tests. Our data therefore suggest that comparisons

of differentiation potential between small sets of PSC lines derived from different individuals will not be a reliable means to identify the impact of genetic variants linked to disease.

T-2398

A SIMPLE TOOL TO IMPROVE THE DIFFERENTIATION POTENTIAL OF HUMAN PLURIPOTENT STEM CELLS

Sundari Chetty, Felicia Walton Pagliuca, Christian Honore, Douglas A. Melton
Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States

Pluripotent stem cell lines show significant variation in their propensity to differentiate, greatly limiting their utility for regenerative medicine and disease modeling. This variation has been attributed to genetic and epigenetic differences between cell lines. In this study, we investigate the role of growth arrest and the cell cycle on differentiation potential and present a different perspective. We show that promoting growth arrest (by culturing pluripotent stem cells in dimethylsulfoxide (DMSO)) prior to directed differentiation can help overcome previously reported barriers and improve multilineage differentiation potential. We show that culturing pluripotent stem cells in DMSO prior to directed differentiation significantly enhances their competency to respond to differentiation signals. DMSO treatment improves differentiation towards all germ layers and leads to enhanced differentiation at later stages of differentiation in more than 25 human embryonic stem cell (hESC) and human induced pluripotent stem cell (hiPSC) lines.

For example, a hES cell line (HUES6) previously shown to have the lowest ectodermal differentiation potential (based on epigenetics and suppressed expression of ectodermal genes) can be “rehabilitated” or “normalized” such that 93% of the cells become ectoderm following DMSO treatment. Similarly, a 0.4% mesodermal differentiation potential is increased to 48% following a DMSO pre-treatment, leading to improvements of more than 100-fold in low propensity cell lines. Furthermore, the DMSO treatment at the pluripotent stage has lasting positive effects on differentiation rates in the subsequent stages of directed differentiation to promote terminal differentiation potential into functional derivates, enabling functionality in cell lines previously considered incompetent. Thus, the DMSO treatment has durable effects and improves differentiation capacity beyond commitment to a given germ layer.

We also demonstrate that the enhanced differentiation potential following DMSO treatment is preceded by activation of the retinoblastoma (Rb) protein and an increased proportion of cells in the G1 phase of the pluripotent cell cycle. While further work is needed to characterize the mechanism through which DMSO operates, our results call for further investigation of the role of Rb and the cell cycle in understanding pluripotent stem cell differentiation. Our findings also suggest that regulating Rb activity using the tools proposed here could have applications in a variety of settings, especially in other contexts where one is trying to manipulate cell fates.

Prior studies have reported that human pluripotent stem cells are restricted in their developmental potential and that only certain cell lines have the capacity to differentiate into particular lineages. Our analysis shows that the developmental potential of pluripotent stem cells is less restricted than previously thought and provides a simple, widely applicable method to improve the differentiation potential and functionality of stem cell lines. The methods developed here permit differentiation of many cell lines toward any desired lineage (implying that one does not necessarily need to pre-select cell lines for differentiation into a particular lineage), and more broadly, improve the prospects of using autologous patient-specific iPS cells for disease modeling and cell replacement therapy.

T-2401

ARF TUMOR SUPPRESSOR AND MIR-205 REGULATE CELL ADHESION AND FORMATION OF EXTRAEMBRYONIC ENDODERM FROM PLURIPOTENT STEM CELLS

Chunliang Li[1], David Finkelstein[2], Charles J. Sherr[1]
[1]*Tumor cell biology, Howard Hughes Medical Institute/St. Jude Children's Research Hospital, Memphis, TN, United States,* [2]*Computational Biology, St. Jude Children's Research Hospital, Memphis, TN, United States*

Induction of the Arf tumor suppressor following oncogene activation engages a p53-dependent transcriptional program that limits the expansion of incipient cancer cells. Although the p19Arf protein is not detected in most tissues of fetal or young adult mice, it is physiologically expressed in the fetal yolk sac, a tissue derived from the extraembryonic endoderm (ExEn). Expression of the mouse p19Arf protein marks late stages of ExEn differentiation in cultured embryoid bodies (EBs) derived from either embryonic stem (ES) cells or induced pluripotent stem (iPS) cells. Arf inactivation delays differentiation of the ExEn lineage within EBs, but not the formation of other germ cell lineages from pluripotent progenitors. Arf is required for the timely induction of ExEn cells in response to Ras/Erk signaling and, in turn, acts through p53 to ensure the development, but not maintenance, of the ExEn lineage. Remarkably, a significant temporal delay in ExEn differentiation detected during the maturation of Arf-null EBs is rescued by enforced expression of miR-205, a micro-RNA up-regulated by p19Arf and p53 that controls ExEn cell migration and adhesion. The noncanonical and canonical roles of Arf in ExEn development and tumor suppression, respectively, may be conceptually linked through mechanisms that govern cell attachment and migration.

T-2402

THE PRESENCE OF PRIMARY CILIA IS REGULATED DURING EARLY MOUSE DEVELOPMENT AND IN STEM CELLS DERIVED FROM EMBRYOS.

Fiona Bangs, Kathryn Anderson
Developmental Biology, Sloan-Kettering Institute, New York, NY, United States

Primary cilia are microtubule-based organelles that project from the cell surface and are required for cells to respond to specific intercellular signals. To determine whether primary cilia have a role in specification of different cell lineages in both stem cell differentiation and during embryonic development, we have generated a double transgenic mouse that expresses Arl13b-mCherry to mark the cilium axoneme and Centrin-GFP to mark the centrioles, which template the cilium. Mouse embryonic stem cells (mESCs) are derived from the inner cell mass (ICM) of the preimplantation embryo at 3.5 days of development. At this stage of development, no cells in the embryo have primary cilia. Over the next two days of development the embryo implants, the ICM proliferates and undergoes dramatic morphogenesis, forming the columnar epithelium known as the epiblast, which is surrounded by extraembryonic endoderm. We first detect primary cilia at 5.5 days of development, but they are present only in the epiblast and not in other lineages of the embryo. This suggests that the presence of cilia is highly regulated, and that cilia may play a role in the ability of cells to respond to the signals that specify early cell lineages. To investigate this further we have made double transgenic ES cells that express both Arl13b-mCherry and Centrin-GFP. As previously reported, we find that ~15% of asynchronously dividing mESCs have a primary cilium. This contrasts both with the absence of cilia on the ICM cells that give rise to mESCs and the postimplantation epiblast, where most cells are ciliated. We are comparing the levels of pluripotency marker expression in non-ciliated verses ciliated mESCs, and whether the level of ciliation changes as cells start to differentiate. This will tell us if primary cilia facilitate cell signaling required to maintain pluripotency or to promote differentiation. Because primary cilia are only present on epiblast cells and not extraembryonic endoderm cells, we are also testing whether primary cilia are present on stem cells derived from each of these two tissues, Epi stem cells derived from the epiblast and XEN cells derived from the extraembryonic endoderm. If Epi stem cells are indeed ciliated, as would be expected since the epiblast has primary cilia, it will be interesting to compare Epi and XEN stem cells as this might provide clues as to what regulates ciliogenesis specifically in the epiblast. Together these studies will help us to understand the role of primary cilia in the early embryo and on stem cells and the role they may play in enabling cells to respond to the signals necessary for specification of different cell lineages.

T-2403

INDUCTION OF DIFFERENTIATION INTO PRIMORDIAL GERM CELL FROM EPIBLAST STEM CELL DERIVED FROM MOUSE EMBRYONIC STEM CELL

Toshiyuki Takehara, Takeshi Teramura, Yuta Onodera, Kanji Fukuda
Institute of Advanced Clinical Medicine, Kinki University Faculty of Medicine, Osaka, Japan

Although several researches for mechanism of germ cell development in mammal are proceeding to date, it mostly remains unclear. Recently, attempts have been made to generate the germ cell or to elucidate the molecular mechanism of gametogenesis by the differentiation induction of germ cell from embryonic stem cell (ESC) *in vitro*. However, because most reports showed that inducing cells had abnormal meiosis and low efficiency, it is still difficult to establish in vitro developmental model by using ESC. In this study, we aimed to induce to primordial germ cell from ESC in vitro culture without adding some cytokines.
As an induction method, we tried to induce by considering *in vivo* environment of embryogenesis. Therefore, the differentiation from ESC to Epiblast stem cell (EpiSC) was used by hypoxia culture condition. Also, to visualize the germ cell marker, we used Oct4-dePE GFP marker. The analysis was performed by Realtime-PCR, Westernblot, Immunofluorescence and FACS analysis.
Firstly, we induced from ESC to EpiSC in hypoxia culture condition. These cells showed disappearance of the Oct4-dePE GFP positive cells. In addition these cells expressed EpiSC marker gene such as *Eomes* and *Lefty1*. Next, we used EB methods in the floating culture. After 2days, the Oct4-dePE GFP positive cell population reemerged at 5% efficiency and these cells expressed the primordial germ cell marker genes such as *Blimp1, Stra8, Dazl* and *Vasa*. Furthermore, these cells exhibited a significant decrease in methylation of imprinting genes such as H19 and Snrpn. Finally, we transplanted the Oct4 dePE-GFP positive cell into the testis of infertility model mouse. After 1 month, the transplantation testis developed further than non-transplantation testis. These testes included the expression of VASA and HASPIN positive cells.
These results indicate that induced cells differentiate to post-migration primordial germ cells which can differentiate to spermatid cells in testis and it is important for inducing condition to consider *in vivo* developmental milieu. Moreover, our research is regarded as one of the research tools to allow the accumulation of knowledge for further progress in infertility medicine and application to regenerative medicine in reproductive
organs.

T-2404

ASTROGLIOSIS PROMOTES FUNCTIONAL RECOVERY OF COMPLETELY TRANSECTED SPINAL CORD FOLLOWING TRANSPLANTATION OF HESC-DERIVED OLIGODENDROCYTE AND MOTONEURON PROGENITORS

Dunja Lukovic[1], Lourdes Valdes-Sanchez[1], Irene Sanchez-Vera[1], Marcela Garita[1], Francisco Diaz-Corrales[1], Victoria Moreno-Manzano[2], Miodrag Stojkovic[3], Shomi S. Bhattacharya[1], **Slaven Erceg**[1]
[1]Cell therapy and Regenerative Medicine, CABIMER, Seville, Spain, [2]Neuroregeneration Lab, Research CEnter, Valencia, Spain, [3]SPEBO, Kragujevac, Spain

Spinal cord injury (SCI) results in neural loss and consequently motor and sensory deficit below the injury. Neural progenitors derived from hESC and iPS cells neural induces locomotor improvement following their transplantation into the animal models of SCI, but little is known about the effects and the underlying mechanism of these grafted cells on local tissue and endogenous neural stem cells. Recently, we have reported the regenerative effects and significant improvement of locomotor function in complete transection rat model of SCI following transplantation of oligodendrocyte progenitors cells (OPC) and motoneuron progenitors (MP) derived from hESC. In the present study, we further analyzed the underlying cell and tissue mechanisms of functional recovery after cell transplantation of OPC and MP hypothesized that beneficial effect is mediated by regenerative signalling pathways activated in the host tissue by transplanted cells. Here we show that transplantation of hESC-derived OPC and MP promote astrogliosis, thorough activation of jagged1-dependent Notch and Jak/STAT signalling supporting axonal survival.

Totipotent/Early Embryo Cells

T-2411

SIGNALLING PATHWAYS THAT DEFINE PLURIPOTENT CELLS IN BOVINE EMBRYOS

Bas Brinkhof[1], Denise Hak[1], Ewart W. Kuijk[3], Leni T.A. Van Tol[1], Henk P. Haagsman[2], Bernard A.J. Roelen[1]
[A]Farm Animal Health, [B]Infectious Diseases and Immunology, [1]Utrecht University, Fac. of Veterinary Medicine, Utrecht, Netherlands, [2]Hubrecht Institute, Utrecht, Netherlands

Embryonic stem (ES) cell lines are derived from the pluripotent cells of a blastocyst stage embryo. Together with the precursors of the primitive endoderm (PE) the pluripotent cells form the Inner Cell Mass (ICM) which, together with the blastocoel, is surrounded by the trophectoderm (TE). Protocols to generate mouse or human ES cell lines have demonstrated to be unsuccessful for the generation of ES cell lines from other mammalian species (e.g. cattle). The generation of ES cell lines from livestock animals might, for example, serve as a source for the generation of cultured meat. The fruitless efforts to establish such cell lines suggest differences between species in the cell signaling pathways responsible for the acquisition or maintenance of pluripotency. One of these pathways is the fibroblast growth factor (FGF)/ mitogen activated protein (MAP) kinase signaling pathway. When the FGF/MAP kinase pathway is activated mouse ES cells start to differentiate while, in contrast, human ES cells are dependent on FGF signaling to remain pluripotent.
We have examined the FGF/MAP kinase signaling pathway in bovine and human blastocyst stage embryos. Similar to mouse embryos, in early cattle and human blastocysts the ICM is composed of NANOG expressing Epiblast precursors and GATA6 expressing PE precursors. When in vitro fertilized bovine embryos were exposed to FGF during embryo culture all cells of the ICM expressed GATA6 indicative of PE precursors. The inhibition of the MAP kinase pathway using the small molecule PD0325901 increased the percentage of NANOG expressing cells in bovine blastocyst stage embryos but, in contrast to mouse embryos, a percentage of the ICM cells remained GATA6 expressing. When human blastocysts were exposed to PD0325901 no change in the percentages of NANOG and GATA6 expressing cells was observed. These results indicate that expression of these transcription factors and developmental fate is regulated via different signaling mechanisms. To elucidate other pathways and mechanisms involved in the formation of pluripotent cells in bovine embryos a microarray-based gene expression comparison has been made between bovine day 5 morulae, intact day 9 blastocysts, isolated TE from day 9 blastocysts and isolated ICM from day 9 blastocysts. After in vitro fertilization of oocytes isolated from slaughterhouse-derived ovaries and in vitro embryo culture until day 9, ICM and TE were mechanically separated. Subsequently, RNA was extracted from 5 batches of ICM, 5 batches of TE, 3 batches of morulae and 3 batches of intact blastocysts. Two batches of RNA from each group totaling more than 10 ng each with RIN values ranging from 8.2 to 9.4 were hybridized on 44K bovine Agilent microarrays. One batch of each group was tested against a common reference pool consisting of RNA isolated from intact blastocysts. To minimize technical error a dye swap experiment was performed using the other batch from each group.
The microarray experiments revealed several differentially expressed genes . The microarray results and the results from the subsequent quantitative PCR analyses will lead to a better understanding of pathways and mechanisms responsible for the acquisition and maintenance of pluripotent cells in cattle embryos and may aid in the establishment of bona fide bovine ES cell lines.

T-2412

NUCLEAR GENOME TRANSFER IN HUMAN OOCYTES TO PREVENT TRANSMISSION OF MTDNA MUTATIONS

Dieter Egli, Daniel Paull
New York Stem Cell Foundation, New York, NY, United States

Mitochondrial DNA (mtDNA) mutations transmitted maternally via oocyte cytoplasm cause numerous debilitating and often fatal mitochondrial disorders. We transferred the nuclear genome between unfertilized oocytes of two different subjects to determine whether the manipulation resulted in a stable exchange of the mitochondrial genotype, and whether the manipulation was compatible with preimplantation development, cellular differentiation,

and nuclear DNA integrity. Nuclear genome transfer allowed efficient development to the blastocyst stage and genome integrity was maintained, provided that spontaneous oocyte activation was avoided through the transfer of incompletely assembled spindle-chromosome complexes. Mitochondrial DNA transferred with the nuclear genome was initially detected at levels below 1% (average 0.3%), decreasing in blastocysts and stem cell lines to undetectable levels (<0.0001%), and remained undetectable after passaging for over one year, clonal expansion, differentiation into neurons, cardiomyocytes or pancreatic β-cells, and after exposing cells to a mtDNA bottleneck during cellular reprogramming. Stem cells and differentiated cells had mitochondrial respiratory chain enzyme activities and oxygen consumption rates indistinguishable from controls. These results demonstrate the potential of nuclear genome transfer to prevent the transmission of mitochondrial disorders in humans.

T-2413

HYPERGLYCEMIA PROMOTES PREMATURE DIFFERENTIATION OF ESCS THROUGH ACTIVATION OF MTORC1 AND SUBSEQUENT AKT INHIBITION

Tiffany Satoorian, Nicole zur Nieden
CBNS, UC Riverside, RIVERSIDE, CA, United States

In the United States, there are 1.85 million women of reproductive age that suffer from diabetes, costing the health care system approximately $1.4 billion dollars annually. Poorly controlled blood glucose (Glu) levels during the first trimester of pregnancy have been shown to result in spontaneous abortion in 20% of diabetic pregnancies, and also impact fertility and blastocyst attachment to the uterine wall. The mechanism by which high blood Glu levels result in these undesired responses is unknown. By discovering the mechanism by which high levels of Glu impact the early embryo, novel drug targets can be identified.
As it is clearly unethical to experiment on human embryos, we have turned to murine embryonic stem cells (ESCs) to study early development in response to hyperglycemia in vitro. These cells are derived from a pre-implantation blastocyst and retain a number of vital characteristics with the early embryo: they are capable of self-renewal, retain the ability to differentiate into cells of the three germ layers, and rely on anaerobic glycolysis as a primary means of adenosine triphosphate (ATP) production. We have previously found that diabetic levels of Glu resulted in a loss in their ability to proliferate rapidly and the ESCs differentiated spontaneously. This was due to the aberrant activation of the transcription factor Forkhead Box O3a (FoxO3a) and transcription of genes that promote differentiation, including the mesodermal marker T-Brachyury. Meanwhile, the FoxO3a inhibitor Akt remained inactive and could not promote removal of FoxO3a from the nucleus. However, the mechanism by which FoxO3a is activated while Akt remains inhibited in response to high Glu conditions is unknown.
Here, we hypothesize that the nutrient-sensor and kinase mammalian target of rapamycin (mTOR) is important for regulating Akt activity. mTOR is the catalytic subunit of two complexes: mTOR complex 1 (mTORC1), which contains the regulatory-associated protein of mTOR (Raptor) and lies downstream of Akt activation, and mTOR complex 2 (mTORC2), which contains the rapamycin-insensitive companion of mTOR (Rictor) and is responsible for Akt activation. We have found that hyperglycemic conditions led to a decrease in Raptor phosphorylation, implying mTORc1 activation. This was accompanied by an increase in Rictor phosphorylation, indicating mTORc2 inhibition. Treatment with the small molecule mTORc1 inhibitor rapamycin in high Glu resulted in colonies that appeared undifferentiated, and there was an increase in Akt phosphorylation. Additionally, while there was a decrease in FoxO3a target gene expression and a decrease in T-Brachyury mRNA and promoter activity, cells were not as proliferative as those cultured in physiological Glu conditions. These results imply that mTORc1 inhibition can partially rescue cells from the premature differentiation observed in hyperglycemia.

Germline Cells

T-2421

HYPOXIA INCREASES PROLIFERATION AND MAINTAINS OCT4 IN ALKALINE PHOSPHATASE POSITIVE MOUSE GERMLINE STEM CELLS THROUGH IGF-IR-HIF-2A CROSSTALK

Yen-Hua Huang[1], Thai-Yen Ling[2], Peng-Cheng Wang[1], Hong-Nerng Ho[3]
[1]Department of Biochemistry, Taipei Medical Univ Sch of Medicine, Taipei, Taiwan, [2]Institute of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan, [3]Graduate Institute of Immunology, College of Medicine, National Taiwan University, Taipei, Taiwan

Recent studies using a transgenic mice model have demonstrated that knockout of hypoxia inducible factor 2α (HIF-2α$^{-/-}$) decreases Oct-4 and primordial germ cell (PGC) survival. However, little is known about hypoxia-interacting endocrinal regulation of PGC proliferation and Oct-4. Herein, we demonstrate a cooperative interaction of HIF-2α and endocrinal insulin-like growth factor-1 receptor (IGF-1R)/Akt/mTOR signaling in proliferation and Oct-4 maintenance of early alkaline phosphatase positive mouse germline stem cells (AP$^+$GSCs). In this study, we demonstrated that hypoxia greatly increased the cell proliferation, up-regulated the expression levels of stemness-related genes, and increased the levels of nuclear Oct-4/HIF-2α protein of AP$^+$GSCs. Additionally, hypoxia significantly increased the expression levels of IGF-1R and secreted-IGF-1; and the IGF-1 dose-dependently increased the HIF-2α expression in AP$^+$GSCs. The inhibition of IGF-IR by RNA interference (shIGF-1R) or PPP (IGF-1R phosphorylation inhibitor) effectively suppressed the IGF-1- and/or hypoxia-induced HIF-2α and Oct-4 expression. The downstream Akt/mTOR signaling involved in the IGF-1/Hypoxia effects. Furthermore, knockdown HIF-2α dramatically suppressed the IGF-IR protein levels in AP$^+$GSC cells. In conclusion, the present study demonstrates a regulatory loop of IGF-1R-HIF-2α in proliferation and Oct-4 maintenance of AP$^+$GSCs under hypoxia. This finding provides insights into the niche endocrinology underlying early germ cell development.

T-2422

EVALUATION OF DIFFERENT FEEDER LAYERS AND CULTURE MEDIA FOR EXPANSION OF OVINE TYPE A SPERMATOGONIA IN VITRO

Uyuanbilig Borjigin[1], Muren Herrid[2], **James McFarlane**[3]
[1]The Key Laboratory of Mammalian Reproductive Biology and Biotechnology, Ministry of Education, Inner Mongolia University, Hohhot, China, [2]Centre for Bioactive Discovery in Health & Ageing, University of New England, Armidale, Australia, [3]Centre for Bioactive Discovery in Health & Ageing, University of New England, Armidale NSW, Australia

Testicular germ cell transplantation techniques have paved the new way in understanding the different aspects of spermatogenesis. Such techniques could potentially lead to the restoration of infertility and the production of transgenic model [1]. Although, marine and human spermatogonial stem cells can be successfully cultured in vitro [2], such a system has not been reported in livestock species yet. In the present study, we have investigated the effect of combination of two types of feeder layer (mouse embryonic fibroblasts (MEF) and Sertoli cells), two types of culture media (αMEM and StemPro-34) and leptin on the culture of ovine type A spermatogonia in vitro. Testicular germ cells were isolated from the testis tissues of prepubertal Merino ram lambs with a scrotal circumference (SC) ranging from 10-15 cm by enzymatic digestion and subjected to differential plating at 37 ºC overnight. Twenty four hours prior to the culture experiment, feeder cells were prepared in a 24 well culture dishes in either αMEM or StemPro-34 supplemented with 5% foetal calf serum (FBS). Enriched type A spermatogonia were seeded in the well at a density of 1 x 104 cells/cm2 in either αMEM or StemPro-34 supplemented with 5% bovine serum albumin (BSA) with or without leptin. The media were changed every 2 days. At the end of 2 weeks culture, colony numbers and cell morphology were recorded. Smears were prepared from the cultures and fixed with 4% paraformaldehyde and then quenched by endogenous peroxidase in Methanol-Peroxide for 5 min. Cells were permeabilized with TBST + 0.001% Triton X-100 and blocked with TBST + 0.1% BSA + corresponding serums for 30 min each. The slides were then incubated with a mixture of mouse anti-PLZF (1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and rabbit anti-VASA (1:10, Abcam, Cambridge, UK) for 30 min. After incubation, slides were rinsed in TBST and a mixture of anti-rabbit Alexa fluor 488 (1:200, Invitrogen) and goat ant-mouse Alexa fluor 594 (1:200, Invitrogen) in TBS with 0.5% BSA was applied for 30 min. In all cultures, cells attached to the dish after 1-2 days of culture and began to form colonies at 3-4 days. The colony number was highest in the αMEM + MEF + leptin (32.1 ± 2.5) culture and was

significantly greater than the lowest in the StemPro-34 + Sertoli cells culture (18.3 ± 3.8). PLZF-VASA double positive cells were detected in all cultures, but the highest number of positive cells (30%) was counted in the the αMEM + MEF + leptin culture. The size of colony in the leptin group in either medium or feeder layers were significantly higher than that of controls. The cell morphology in the leptin groups also appeared to be healthier than those in the controls.
In summary, the combination of αMEM and MEF appears to be more suitable for culture of ovine type A spermatogonia in vitro, while leptin has an effect in promoting cell survival.
Reference
[1]. Brinster RL and Zimmermann JW. Spermatogenesis following male germ-cell transplantation. Proc Natl Acad Sci USA. 1994, 91(24):11298-302.
[2]. Brinster RL. Male germline stem cells: from mice to men. Science. 2007, 316:404-5.

T-2423

MOLECULAR AND FUNCTIONAL COMPARISON OF SPERMATOGONIAL CELL SUBPOPULATIONS IN THE DOMESTIC CAT

Robin H. Powell[1], Monica N. Biancardi[2], Jason L. Galiguis[2], Qian Qin[2], C. E. Pope[2], Stanley P. Leibo[1], Guoshun Wang[3], Martha C. Gomez[2]
[1]Biological Sciences, University of New Orleans, New Orleans, LA, United States, [2]Audubon Center for Research of Endangered Species, New Orleans, LA, United States, [3]Gene Therapy, Louisiana State University Health Sciences Center, New Orleans, LA, United States

Most
of the 36 wild felid species are listed as threatened or endangered, and
attempts to promote reproduction through captive breeding and assisted
reproduction techniques have varied success rates. Spermatogonial stem cells (SSCs), comprising
only a small percentage of germ cells which reside in the seminiferous tubules,
have the dual ability to self-renew and differentiate into spermatozoa
throughout the life of the male. SSC transplantation
may be a useful method in perpetuating valuable males as demonstrated by the
production of ocelot sperm following ocelot SSC transplantation to domestic cat
testes (Silva *et*
al. 2012 *J Androl.*; 33, 264-76).
Although transplantation of mixed germ cell populations has been
successful in some mammalian species, purified SSCs via SSC surface markers may
lead to better success rates in animal cloning and a better understanding of
germ cell biology. We previously
reported that SSC marker GFRα1 was detected in spermatogonia as well as more
differentiated germ cells in the cat, but pluripotent markers stage-specific
embryonic antigen 1 (SSEA-1) and SSEA-4 were specific to spermatogonia in both
pre-pubertal and adult testes, although SSEA-4 + cells were in lower number (Powell *et*
al. 2011 *Reprod. Fertil. Dev.*;
24, 221-22; Powell *et al. 2012 Reprod. Fertil. Dev.*; 25, 290-1). Double
staining of GFRα1 and SSEA-4 showed distinct
cell populations that stained positively for GFRα1, a subpopulation of which
co-localized with some SSEA-4 + cells, and SSEA-4 + only, reflecting the
undifferentiated stage of cat SSCs. The present goal was to isolate cat SSCs by flow
cytometry with SSC surface markers and compare the expression of pluripotent
genes in each cell subpopulation to identify which population highly expresses
the genes of interest. Mixed germ cells

from 10-12 adult testes were pooled, single-stained with SSEA-1 or SSEA-4 or
double-stained with GFRα1 and SSEA-4, followed by flow cytometry sorting. The relative transcript abundance for the
pluripotent genes *Nanog*, *POU5F1*, and *Sox2* in each subpopulation was subsequently examined by Rt-qPCR. As compared to the non-sorted control, cat SSCs
positive for SSEA-1 only expressed high levels of pluripotent gene *Nanog*.
In contrast, SSCs positive for SSEA-4 expressed no *Nanog*, but low levels of *POU5F1*
and *Sox2*. Cells positive for both GFRα1 and SSEA-4
expressed lower levels of the three pluripotent markers than the non-sorted
cells, while cells sorted for GFRα1 or SSEA-4 alone did not express any of the
genes. In summary, both SSEA-4 and
SSEA-1 are good markers for identifying and isolating cat SSCs. The biological differences between cell populations require further investigation.

T-2424

MOUSE OVARIAN VERY SMALL EMBRYONIC-LIKE STEM CELLS RESIST CHEMOTHERAPY AND UNDERGO OOGENESIS IN VITRO IN RESPONSE TO FOLLICLE STIMULATING HORMONE

Kalpana Sriraman, Deepa Bhartiya, Smita Bhutda, Sandhya Anand
Stem Cell Biology, National Institute for Research in Reproductive Health, Mumbai, India

The long-held dogma in female ovarian biology is that women and other mammalian females are born with fixed and non-renewing pool of germ cells. This doctrine has been challenged in recent years by several researchers and strong evidence for post-natal oogenesis has emerged. Recently, Dr Tilly's group have isolated and propagated purified ovarian stem cells from mouse and human ovary and also demonstrated its potential to form oocytes both *in vitro* and *in vivo*. Our group has shown that mammalian ovarian surface epithelium harbors two types of stem cells-very small embryonic-like stem cell (VSELs) and larger progenitor stem cells -the ovarian germ stem cell (OGSCs). The main aim of this study was to investigate the effect of chemotherapy on these stem cells in mouse ovaries and further evaluate their differentiation potential *in vitro*.
8-weeks old Swiss mice were treated with busulphan (10mg/Kg body weight) for four days along with cyclophosphamide (100mg/Kg body weight, ip) on first two days. One month after the treatment, the ovaries were analyzed by histology, Q-PCR for gene expression levels and immunolocalization. The ovaries were also cultured as whole ovary organ cultures with and without follicle stimulating hormone (FSH; 10mIU/ml) to investigate the differentiation potential.
Histological and germ cell specific gene expression analysis showed almost complete loss of follicles and germ cells in ovaries collected from treated animals confirming treatment-induced infertility. Interestingly, immunolocalization studies using stem cell specific markers OCT-4, SSEA-1 and SCA-1 showed that VSELs in mouse ovary are resistant to combined busulphan and cyclophosphamide treatment, while progenitor OGSCs and follicles in various developing stages were adversely affected. This correlated well to gene expression analysis for *Oct-4* and *Oct-4A*. These stem cells were unable to differentiate post-treatment probably due to compromised niche.
Further *in vitro* culture of germ cell ablated mouse ovaries were done to investigate potential differentiation of persisting VSELs into oocyte. We have observed that culturing in presence of FSH leads to up-regulation of *Oct*-4, *Sca-1* and *DazL* (germ cell marker that is known to play critical role in germ cell development and differentiation) suggesting increase in stem cell activity and differentiation. Histological analysis showed new germ cell nest like structures that stained positive for Mouse Vasa Homolog, a germ cell marker. These results suggest that resistant stem cells can overcome differentiation block upon culturing in presence of FSH and undergo proliferation and differentiation to form new germ cells/oogonia.
To conclude, mouse ovarian VSELs resist and survive chemotherapy while the progenitors, oocytes, follicles and somatic niche are adversely affected. These VSELs show potential to differentiate into oocytes *in vitro* probably due to more conducive environment. This study supports the concept of post-natal oogenesis and also opens up new possibilities for treatment of infertility in cancer survivors.

T-2431

IDENTIFICATION OF NEW PORCINE SPERMATOGONIAL STEM CELL MARKERS USING TOTAL TRANSCRIPTOME ANALYSIS

WonYoung Lee, Ran Lee, KyungHoon Lee, HeeChan Kim, KyungSook Nho, Hyuk Song
Department of Animal & Food Bioscience, College of Natural Science, KonKun University, Chungju, Korea, Republic of

Identification of molecular markers for specific cells in developmental stage is critical to characterize and study of stem cells. Recently our group established a porcine spermatogonial stem cells (pSSCs) line using low temperature culture condition, however lack of specific markers for pSSCs restrict the further research of this cells. In this study, total transcriptome analysis was performed to identify the pSSCs specific markers. Total transcriptome among the established pSSCs, day 5 and 180 porcine testis were compared using RNA-sequencing protocols. Among the 10,184 genes, differentially expressed genes having more than 10 fold changes were selected using Cufflink S/W. As a result, 121 genes were selected from the comparing in between pSSCs with 5 day testis, 863 genes were selected from the comparison between pSSC and 180 day testis. Among the differentially expressed genes, 67 genes were up-regulated and 54 genes were down-regulated in 5 day testis compared to pSSCs. Interestingly, CD14, CD209, KLF9, IGFBP3 and IGFBP5 were strongly up-regulated in *in vitro* cultured pSSC lines. The RT-PCR and western blotting of these molecules revealed that these molecules were expressed specifically in pSSCs. The immunocyto- and histochemistry data also support that these molecules are putative markers for pSSCs. In conclusion, CD14, CD209, KLF9, IGFBP3 and 5 are preferentially expressed in porcine spermatogonia stem cells, and these molecules can be the putative markers for pSSCs. This finding may facilitate the study on spermatogenesis and further application of spermatogonial stem cells.

T-2432

IN VITRO RECONSTRUCTION OF MOUSE SEMINIFEROUS TUBULES SUPPORTING GERM CELL DIFFERENCIATION

Tetsuhiro Yokonishi, Takuya Sato, Kumiko Katagiri, Mitsuru Komeya, Yoshinobu Kubota, Takehiko Ogawa
Department of Urology, Yokohama city university, Yokohama, Japan

It is known that cells of testis tissues in fetal or neonatal periods have the ability to reconstruct the testicular architecture even after dissociation into single cells. This ability, however, has not been demonstrated effectively *in vitro*. In our present study, we succeeded in reconstructing seminiferous tubules *in vitro* which supported spermatogenesis to meiotic phase. Testis cells of neonatal mice were dissociated enzymatically into single cells. The cells formed aggregates in suspension culture and were transferred to the surface of agarose gel to continue the culture with a gas-liquid interphase method, where a tubular architecture gradually developed during the following 2weeks. Immunohistological examination confirmed Sertoli cells forming tubules and germ cells inside. With testis tissues of *Acr*-GFP transgenic mice, whose germ cells express GFP during meiosis, cell aggregates formed a tubular structure and showed GFP expressions in their reconstructed tissues. Meiotic figures were also confirmed by regular histology and immunohistochemistry. In addition, we mixed cell lines of spermagonial stem cells (GS cells) into the testis cell suspension, and found the incorporation of GS cells in the tubules in reconstructed tissues. When GS cells derived from *Acr*-GFP transgenic mice were used, GFP expression was observed, indicating that the spermatogenesis of GS cells was proceeding up to the meiotic phase. This *in vitro* reconstruction technique will be a useful method for the study of testis organogenesis and spermatogenesis.

T-2433

MOLECULAR CHARACTERIZATION AND CULTURE OF TESTICULAR GERM CELLS IN NON-HUMAN PRIMATES

Zachary YC Lin[1], Masanori Imamura[1], Takamasa Hirano[2], Eiji Matsunaga[3], Miki Taoka[3], Hiroo Imai[4], Hirotaka J. Okano[1], Atsushi Iriki[3], Mikiko C. Siomi[5], Haruhiko Siomi[2], Erika Sasaki[6], Hirohisa Hirai[7], Hideyuki Okano[1]

[A]Physiology, [B]Molecular biology, [1]Keio University, Tokyo, Japan, [2]Laboratory for Symbolic Cognitive Development, RIKEN BSI, Tokyo, Japan, [3]Primate Research Institute, Kyoto University, Nagoya, Japan, [4]Biophysics & Biochemistry, Tokyo University, Tokyo, Japan, [5]Central Institute for Experimental Animals, Tokyo, Japan, [6]Primate Research Institute, Kyoto University, Tokyo, Japan

Germ cell development is a fundamental process to produce offspring and sustain the species in multicellular organisms. In the course of evolution, the detail developmental program of spermatogenesis has become distinctive in each species. The interspecies difference has prevented simple application of rodent insights to higher primate, despite the accumulated knowledge on the molecular properties of germ cells in the mouse. Hence, considering an extrapolation to human, it is essential to investigate the nature of germ cells in non-human primate rather than rodent. In this study, we utilized common marmoset (Callithrix jacchus) as an animal model for dissecting the molecular properties of primate spermatogenic cells as well as for developing the novel culture system of them.
To determine the molecular signatures of marmoset spermatogenic cells, we have examined gene expression and DNA methylation status according to the previous studies on rodent and human. Eventually, we have discovered marmoset-specific features distinct from rodent or human, which became an important platform for designing the culture system of marmoset spermatogenic cells. Then, we next established a novel culture method for marmoset testicular germ cells in the form of cell aggregates named “testicular sphere”. By using this method, we could cultivate testicular germ cells for more than 4 weeks without losing the alkaline phosphatase activity. RT-PCR and immunohistochemistry suggested that testicular spheres contained spermatogonial cells but did not induce de novo spermatogenesis in vitro. We also confirmed the feasibility of lentiviral gene transduction and cryopreservation of testicular spheres.
Albeit many advantages of marmoset as an experimental animal for a non-human primate model, we further aimed to apply our methodology to other higher primate species, such as Japanese macaque in Old-World money and chimpanzee in Great Ape, phylogenically much related to human. The preliminary data showed that both Japanese macaque and chimpanzee testicular germ cells could form testicular spheres with alkaline phosphatase activity, similar to those of marmoset. Additionally, we are now constructing in vitro germ cell differentiation approaches. We believe that our study could bring us an important insight for the primate germ cell biology.

Pre-clinical and Clinical Applications of Mesenchymal

T-3001

THE BONE MARROW-DERIVED MESENQUIMAL STEM CELLS REPAIR THE ACUTE KIDNEY INJURY INDUCED BY ACYCLOVIR.

Joelma S. Christo, Paulo M. Lopes, Luciana A. Reis, Manuel J. Simões, Nestor Schor
Nephrology Division-Medicine Dept, Escola Paulista de Medicina/UNIFESP, Sao Paulo, Brazil

Introduction: Acyclovir (Acy) is an antiviral drug used to treat herpes simplex type 1 and 2 and varicella zoster. This drug is widely distributed in all body tissues, being very high in the kidneys, which may induce nephrotoxicity characterized by an acute kidney injury (AKI), with increases in creatinine and urea resulting from the reduction in the glomerular filtration and acute tubular necrosis. Several groups have reported the contribution bone marrow-derived mesenquimal stem cells (BMSCs) in repair processes using different animal models. The aim of this study was to investigate if BMSCs can attenuate tubular damage in this nephrotoxic model of AKI. Methods: BMSCs were characterized by FACS analysis and differentiation into adipocytes and osteocytes. The cells were cultured and employed at 4th passages for all experiments. Female Wistar rats received Acy (80mg/Kg/BW) (Acy group) or water (CTL) ip, N=10 for each group, during 5 days. After 48 or 72 hours, the female rats received iv BMSCs (1X106-cells). Then, blood and 24 hours urine were collected for creatinine (Cr) and urea (U evaluations. The animals were sacrificed and kidneys were perfused and removed for histology. It was observed that Cr (1.7± 0.1 mg/dl) and U (174.5±0.2 mg/dl) were higher in Acy group when compared to CTL (0.7±0.01 and 56.0±0.1 mg/dl, respectively, $p<0.05$) after 5 days of Acy treatment. For both Acy+BMSCs groups (48 and 72 hours) it was observed lower Cr and U serum values compared with Acy group alone: After 48 hours ~30% and ~40% for Cr and U and after 72 hours

~40% and ~35% ($p<0.05$) lower values were observed with BMSCs, respectively. At the end of 5 days of Acy treatment it was observed glomerular congestion, cell damage, tubular dilation and pyknotic nuclei. However, for The Acy+BMSCs groups, after 48 and 72 hours of BMSCs transplantation, histological analysis pointed that both glomerular and tubular effect of Acy are much less impressive by qualitative evaluation.
Supported by FAPESP, CNPq, CAPES and FOR

T-3002

MESENCHYMAL STEM CELL EXOSOMES TRANSFER microRNA AND STIMULATE THE PROLIFERATION OF INJURED TUBULAR EPITHELIAL CELLS.

Fernanda T. Borges, Luciana A. Reis, Nestor Schor
Nephrology Division-Medicine Dept, Escola Paulista de Medicina/UNIFESP, Sao Paulo, Brazil

Bone Marrow Mesenchymal Stem Cells (BM-MSCs) protect the kidney against nephrotoxic insults through paracrine effects, possibly mediated by extracellular vesicles. Exosomes (EXOs) are up to 100nm extracellular vesicles that transfer mRNA and microRNA to the recipient cells, mediating the communication between them. MicroRNA 23a, 126 and 296 were shown to mediate the protective effect of stem cell microvesicles. The goal of the present study was to analyze the effects of EXOs from MSCs on human tubular epithelial cells (HK-2) exposed to toxic insults. MSCs were maintained in culture conditions and every 3 days the culture medium was collected and EXOs were extracted through filtration in 0.1µm filter, followed by ultracentrifugation's (100,000g during 2h). 23a, 126 and 296 microRNAs from MSCs and EXOs were extracted and analyzed by real time-PCR. HK-2 cells were exposed to gentamicin (Genta, 2mM) in the presence or absence of exosomes (50 µg/ml) and proliferation was analyzed from 24 up to 96h through MTT assay. MSCs were instable transfected with EXOs marker GFP-CD63, cultured on the top of an insert. On the bottom of the insert, tubular cells were treated with Genta. After 24h, the fluorescence of GFP was observed on HK-2, suggesting that EXOs were taken up by tubular cells. Related to the endogenous control, the microRNA 23a (EXOs: 3.18 ± 0.85 versus MSCs: 0.39 ± 0.05 arbitrary units), 126 (EXOs: 2.80 ± 0.87 versus MSCs: 0.16 ± 0.03 arbitrary units) and 296 (EXOs: 0.44 ± 0.08 versus MSCs: 0.004 ± 0.001 arbitrary units) were more expressed in the exosomes than MSC cells. The microRNA 23a and 126 were more expressed than 296 in EXO. The treatment with EXOs stimulated the proliferation of HK-2 exposed to Genta after 72 (EXOs+Genta: 0.39 ± 0.01 versus Genta: 0.23 ± 0.2 Arbitrary Units) and 96 h (EXOs+Genta: 0.37 ± 0.01 versus Genta: 0.25 ± 0.2 Arbitrary Units). Our results suggest that EXOs carrying microRNA from MSCs are taken up and stimulated the proliferation of tubular epithelial cells, mediating the proximal tubule regeneration.
Supported by FAPESP, CNPq, CAPES and FOR

T-3003

CELL AND GENE THERAPY TO MITIGATE CUTANEOUS RADIATION SYNDROME : EVALUATION IN ANIMAL MODELS

Diane R. Riccobono[1], Agay Diane PhD[1], fabien Forcheron[1], Harry Scherthan[2], Viktor Meineke[2], Michel Drouet[1]
[1]Radiobiology, French Armed Biomedical Research Institut, La Tronche Cedex, France, [2]Radiobiology, Institut fur Radiobiology der bundeswehr, Munchen, Germany

Accidental or therapeutic localized irradiation at high doses frequently results in Cutaneous Radiation Syndrome (CRS) characterized by inflammatory waves, poor revascularization and incomplete healing. Nowadays, local injection of autologous Bone-Marrow Mesenchymal Stem cells (BM-MSCs) is part of CRS treatment. However its efficacy may be hampered by lack of available cells -duration of culture or delayed diagnosis. In this context the development of allogeneic MSCs banks, the use of conditioned media (CM) or new stem cell sources such as adipocyte derived stem cells (ASCs) as well as the improvement of MSC secretome -i.e transient gene therapy- may represent promising strategies. Our group has set up different animal models in order to clarify these points.
First the therapeutic potential of subcutaneous injection of allogeneic versus autologous ASCs (50x106 each animal) has been evaluated in Göttingen minipigs: local irradiation at 50Gy gamma 60Co; for each group n=5; phosphate buffered saline controls n=8. Clinical follow up showed a significant improvement of wound healing in animals given

autologous ASCs in whom histological staining confirmed keratinisation and neovascularisation (Von Willebrandt factor staining). In contrast animals injected with allogeneic ASCs and controls developed severe necrosis.
Second to optimize the secretome ASCs were electroporated with a PIRES2 bicistronic plasmid coding for the pro-angiogenic Sonic hedgehog (Shh) morphogene. The capacity of ASCs or Shh-ASCs CM to modulate the response of 25Gy in vitro irradiated fibroblasts was evaluated in short term cultures according to the following criteria: apoptosis (annexin V staining; n=9), senescence (β-galactosidase staining; n=9) and pro-angiogenic gene expression (VEGFα and angiopoïetin 1 (ANGPT1) using RTqPCR (n=3)). CM from Shh-ASCs significantly decreased cellular death from 6 to 24 hours (H) post irradiation and senescence at H48. Furthermore, fibroblasts expression of VEGFα and ANGPT1 was increased in presence of both CMs at H24. In a supplementary model BD Matrigels® "growth factors reduced" loaded or not with ASCs or Shh-ASCs were implanted subcutaneously in immunocompromized mice (n=7 for each group). 14 days later, mice were euthanized and haemoglobin Matrigels® content was quantified by spectrophotometry using Drabkin's method. A four-fold increase was observed in Shh-ASC Matrigel compared with Matrigel alone ($p<0,01$).
Finally autologous (n=2) or allogeneic (n=1) Shh-ASCs were locally injected in 50Gy irradiated minipigs. Injection was well tolerated. Preliminary data suggest that the "allogeneic" pig did not differ from controls and that for "autologous" pigs the cell dose may represent a crucial parameter.

T-3004

ENABLING AUTOLOGOUS HUMAN LIVER REGENERATION WITH DIFFERENTIATED ADIPOCYTE STEM CELLS

Dan Xu, Gary Peltz
Anesthesia, Stanford University, Stanford, CA, United States

Using procedures that are scalable and appropriate for human clinical use, we have developed methodology that makes it possible to regenerate human liver using autologous adipocyte-derived stem cells (ASC). We developed a novel method, which involves culture in chemically defined media for a short period of time, for efficiently inducing ASC to differentiate into induced hepatocyte-like cells (SCi-Heps). We demonstrate that SCi-Heps have many of the functional properties of mature hepatocytes in vitro, and that they can reconstitute functioning human liver in vivo, with stable biosynthetic function, in a recently developed murine model system after ultrasound-guided direct injection into liver. Implantation studies demonstrate that SCi-Heps have a very low malignant potential. Since the ASC were obtained using a commonly performed clinical procedure (liposuction), these methods can be used to achieve the major goal for regenerative medicine: regenerating human liver using autologous stem cells obtained from a readily accessible tissue.

T-3005

IN SITU REPROGRAMMED SPERMINE TREATED ADIPOSE TISSUE DERIVED MULTI LINEAGE PROGENITOR CELLS IMPROVE LEFT VENTRICULAR DYSFUNCTION IN A SWINE CHRONIC MYOCARDIAL INFARCTION MODEL

Hanayuki Okura[1], Mayumi Soeda[1], Shigeru Miyagawa[2], Yoshiki Sawa[2], Akihiro Ichinose[3], Akifumi Matsuyama[1]
[1]Foundation for Biomedical Res & Innovation, Kobe, Japan, [2]Osaka University Graduate School of Medicine., Osaka, Japan, [3]Kobe University Hospital, Kobe, Japan

Background: Spermine, known as one of polyamines, has been reported to make embryonic stem cells differentiate into cardiac lineage. In this study, we examined whether spermine could commit human adipose tissue-derived multi-lineage progenitor cells (hADMPCs) into cardiac lineage and whether the spermine treated-hADMPCs would differentiate into cardiomyocytes-like cells and improve left ventricular dysfunction in a swine chronic myocardial infarction model. Methods and Results: After 24h-treatment with spermine, hADMPCs showed the augmentation of cardiac marker-expressions; nkx2.5, islet-1, alpha-cardiac actin and cardiac troponin I (11.2-, 27.5-, 43.6- and 19.1-fold to hADMPCs per se, respectively). To examine the effect of spermine treated-hADMPCs on left ventricular dysfunction, swine chronic MI model were built up by first ballooning and reperfusion to first diagonal branch and second one to left ascending coronary artery (#6) 1 week-later. Four week-later second one, the swine (immuniz-

ation with CyA 0.6mg i.m./kg/day) received transplantation of spermine treated-hADMPCs (1x105, 3x105, 1x106 and 3x106 cells/kg) or lactic Ringer's solution via intracoronary (#6), and echocardiogram was examined at 0, 4, 8 and 12 weeks after transplantation. Follow-up showed rescue of function in the transplanted, and the most effective dose was 3x105 cells/kg (EF; 33.4%, 47.0%, 51.5% and 52.9% at 0, 4, 8 and 12 week-after transplantation, respectively). Histologically, the spermine treated-hADMPCs were engrafted into the scarred myocardium and reprogrammed into human specific troponin I and alpha-cardiac actin positive cells in situ 12 week-after transplantation. Conclusion: The transplantation of spermine treated-hADMPCs is a potentially effective therapeutic strategy for future cardiac tissue regeneration.

T-3006

ADIPOSE TISSUE DERIVED STEM CELL THERAPY FOR ACUTE HIND LIMB ISCHEMIA TREATMENT

Ngoc Bich Vu, Lan Thi Phi, Van Ngoc-le Trinh, Ngoc Kim Phan, **Phuc Van Pham**
Laboratory of Stem Cell Research and Application, Univ of Science, VNU-HCM, Ho Chi Minh, Viet Nam

Limb ischemia is a common disease that occurs when there is a sudden lack of blood flow to a limb due to embolism or thrombosis. Currently, treatment of this disease is extremely difficult and shows poor efficacy. De novo angiogenesis and vasculogenesis based on stem cell therapy is considered as a promising therapy for treating this disease. Therefore, this study aimed to evaluate the safety and efficacy of adipose tissue derived stem cell (ADSC) transplantation to treat acute limb ischemia in mouse models. Adipose tissue was collected from murine abdomen for auto-graft. ADSCs were derived the adipose tissue by ADSC Extraction kit using collagenase I and II to digest the tissue. ADSCs were cultured in DMEM/F12 supplemented with 10% FBS (fetal bovine serum) and 1% antibiotic-mycotic. Obtained ADSCs were confirmed by specific marker expression and in vitro differentiation into adipocytes and osteoblasts. Acute hind limb ischemic mouse models were established by femoral artery ligation following published protocols. Acute hind limb ischemic mouse models were treated with ADSCs by three methods: injection into the ischemic region, injection into the tail vein, and a combination of both. The treatment efficacy was evaluated by the degree of ischemic damage, tissue edema, neuromuscular scores, pedal reflex frequency, and histology. The results showed that ADSCs significantly improved hind limb ischemia. ADSC transplantation assisted recovery of the ischemic tissue from grade IV to III, II, I and 0, with the best outcome achieved with combinatorial therapy in which 6/10 treated mice returned to normal (Grade 0). These results indicate that ADSCs is a promising source to isolate stem cells for hind limb ischemia treatment.

T-3007

TREATMENT OF HIP DYSPLASIA IN DOGS WITH ACUPOINT INJECTION OF AUTOLOGOUS OR ALLOGENEIC ADIPOSE-DERIVED STEM CELLS

Isabel Selbach[1], Maiele D. Silveira[2], Lucas P. Cardoso[2], Luisa M. Braga[2], Melissa Camassola[3], **Nance B. Nardi**[3]
[1]Clinivetis, Novo Hamburgo, Brazil, [2]CellVet Medicina Veterinaria Regenerativa, Canoas, Brazil, [3]Universidade Luterana do Brasil, Canoas, Brazil

Adipose-derived stem cells (ADSC) show great therapeutic potential in treating degenerative diseases. Since 2003, ADSC have been employed in veterinary medicine, mainly for bone, tendon and ligament injuries and joint diseases. However, some points should be better explored, such as the use of fresh or cultured cells, number of cells used and route of administration. This study aimed to evaluate the efficacy of autologous stromal vascular fraction (SVF) or allogeneic cultivated ADSC injected into acupuncture points in dogs with hip dysplasia. Three male and six female dogs of different breed, ranging in age from 1 to 10 years and weighting 30-35 kg, were included in the study. All dogs had hip dysplasia and weak respond to drug therapy. Before inclusion, the owners agreed by informed consent to participate in the clinical study and to observe their dog during the study period. All dogs underwent routine

clinical and hematological evaluation to ensure overall health, and any previous lesion was assessed by clinical and RX examination. A veterinarian assessed the dogs for pain in manipulation, alteration of range of motion and functional disability. Adipose tissue (5 - 10 g) was collected from the inguinal region, using standard surgical procedures and a mild anesthesia. The tissue was digested with collagenase type I and the SVF (5x106 cells/animal) was used for treating the donor dog (n=4). Canine ADSC cultures were established, characterized for proliferation and differentiation potential and used for treating the remaining 5 dogs (106 cells/animal). The cells were injected in 3 points of acupuncture (Bladder 54, Gall bladder 29 and Gall bladder 30) near the joint. Analyses of ADSC cultures showed normal morphology and proliferation capacity, and the cells were able to differentiate into adipocytes, osteoblasts and chondrocytes. After the first week of treatment, clinical examination of all patients showed marked improvement compared with baseline results. One month later, all dogs showed improvements in lameness at trot, as well as in pain on manipulation of the joints. According to the owners assessment, outcomes also improved markedly. These results show that canine ADSC show proliferation and differentiation potential similar to ADSC from other species. Allogeneic ADSC and autologous SVF showed similar therapeutic potential in hip dysplasia in dogs, when administered in acupuncture points, and represent an important therapeutic alternative for this type of pathology.

T-3008

HUMAN AMNIOTIC FLUID: A POTENTIAL SOURCE OF MULTIPOTENT STEM CELLS

Zhisheng Chen[1], Fang Wang[2], Michael Chan[3], MaryAnn Perle[4], Ross Basch[4], Bruce Young[3]
[1]Foshan University, Foshan, Guangdong Province, China, [2]Obstetrics and Gynecology, New York University Medical Center, ,, New York, NY, United States, [3]Obstetrics and Gynecology, New York University Medical Center, New York, NY, United States, [4]Pathology, New York University Medical Center,, New York, NY, United States

Human Amniotic Fluid: A source of a variety of multipotent stem cells for clinical use

Abstract
Amniotic fluid cells from second trimester amniocentesis have been found to be a source of multipotent stem cells which might overcome the limitations of expansion, histocompatibility, tumorigenesis and ethical issues associated with the use of human embryonic cells. Previous work by DeCoppi demonstrated the pluripotency and growth patterns in c-Kit selected cells. We sought to perform a more comprehensive investigation of the pluripotency of human amniotic fluid -derived stem cells (hAFSC) and quantify the culture characteristics and distribution of stem cells markers in c-Kit selected cells compared to c-Kit negative cells. We found less than 5% of HAFSC were c-Kit positive. However when cultured between 15-90% of the c-Kit negative cells expressed CD90, SSEA4 or TRA-1-60, in varying amounts . There was persistence of stem cell markers including expression of SSEA4, TRA-1-60, CD90 in vitro through multiple passages and subpopulations in a high percentage of cells. But telomere lengths of HAFSC are similar to fibroblasts, much shorter than hES cells. This may be part of the reason for the relatively limited life span of cultured HAFSC, averaging about 16 passages before senescing. There is significant(is this so? Was it a statistically significant difference?) increase in Oct4, Nanog and Sox2 gene mRNA expression in cells derived from 15-17 gestation week amniotic fluid samples versus the longer gestational age groups. Double and triple labeled cell populations were identified by MACS .
We showed that 5.5% of c-Kit negative cells are triple positive for SSEA4, TRA-1-60 and CD90 expression. This may be a more efficient method than c-kit selection of hAFSC because their stem cell markers expression was equal to or exceeded by the c-kit negative cells in our results. Our results add to and confirm those of others that human amniotic fluid derived cells maintain stem cell pluripotency markers in culture over enough passages to provide sufficient numbers of cells for possible clinical use. The occurrence of triple-labeled cell populations poses the intriguing possibility of cells with a closer resemblance to embryonic stem cells, or with a greater potential to differentiate into a variety of specific lineages.
Figure 1. Comparison of proliferation potential in 4 groups by gestation week.Figure 2. Stem cell marker Oct-4, Nanog, and Sox2 gene mRNA expression are significant increased in 15-17 gestation week samples.

T-3011

CELL FUSION PHENOMENA PRESENTS AFTER PRENATAL TRANSPLANTATION OF DS-RED PORCINE AMNIOTIC FLUID STEM CELLS

Steven Shaw[1], SHAO-YU PENG[2], PO-JEN CHENG[1], WINSTON TK CHENG[3], Shinn-Chin WU[3]
[1]Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, Taoyuan, Taiwan, [A]INSTITUTE OF BIOTECHNOLOGY, [B]Department of Animal Science and Technology, [2]NATIONAL TAIWAN UNIVERSITY, TAIPEI, Taiwan

Objectives: Amniotic fluid stem cells (AFSCs) is known to derived from amniotic fluid of developing fetus can give rise to diverse differentiated cells including ectoderm, mesoderm and endoderm lineages. Intra-uterus transplantation (IUT) is an approach to cure inherited genetic defects for fetuses during the gestation period of pregnant dams. Certain diseases such as osteogenesis imperfect or muscular destrophy were successfully treated to ameliorate the syndrome. However, the donor cell destiny is still uncertain.
Methods: The purpose of this study was to investigate the bio-distribution and the cell fate of Ds-red harboring porcine amniotic fluid derived stem cell (Ds-red pAFSCs) after intra-uterus transplanting into EGFP-harboring fetus of pregnant mice. The 12.5 days pregnant mice were performed the IUT surgery by pAFSCs injection.
Results: Three weeks after birth, the mice (N=12) were sacrificed. Several portions from different organs were sampled for histological examination and flow cytometry analysis. Experiment results showed that migration of Ds-red pAFSCs was expressed most significantly in intestines of the mice (16.5%). Furthermore, EGFP signal and RFP signal were co-expressed in the intestine and liver cells observed by immunostaining.
Conclusion: It is concluded that the exogenous implantation of pAFSC was fused with recipient intestinal cells instead of differentiation or maintaining the undifferentiated status in the tissue and could be used as the source of cell therapy.

T-3012

AUTOLOGOUS BONE MARROW DERIVED MESENCHYMAL STROMAL CELL THERAPY IN AMYOTROPHIC LATERAL SCLEROSIS: A SAFETY STUDY

Seung Hyun Kim, Chanil Moon, Ki-wook Oh, Seong-il Oh, Won Jun Chol, Kyung Sook Kim, Joon Ho Lee
Hanyang University Seoul Hospital, Cell Therapy Center for Neurologic disorders, Seoul, Korea, Republic of

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by involvement of motor neurons in the cerebral cortex, brainstem and spinal cord. The underlying cause of the disease remains unclear, and the disease usually progresses to death within 2 to 4 years. There is no effective treatment and many previous efforts using various neuroprotective agents according to a variety of proposed pathogenic mechanism in ALS did not prove successful. Recently, the use of multipotent mesenchymal stem cells/marrow stromal cells (MSCs) represent a promising tool for stem cell-based therapeutics in ALS. Many effects produced by the MSCs are explained by paracrine mechanisms, including a potent anti-inflammatory capacity, the direct release of anti-apoptotic and neuroprotective factors, and the ability to induce proliferation of local neural progenitor cells. Also MSCs possess immunomodulating properties on cell populations of both adpative and innate immunity. These effects of MSCs might be beneficial in ALS. In previous our animal study and pilot clinical study, we evaluated the dose dependent effects of MSCs, and a 1 X 10^6 cells significantly prolonged life span and slowed disease progression. On the basis of the preclinical animal study and the data from pilot clinical studies, we initiated an exploratory trial to assess safety of autologous bone marrow-derived MSCs into intrathecal space in patients with ALS.
This study, designed as a phase 1 open, single-arm, single dose, safety clinical trial, was approved by the Institutional Review Board of the Hanyang University Hospital, and Korean Food and Drug Administration(ClinicalTrial.gov ID. NCT01363401). 7 Consenting patients (4 men, and 3 women) fulfilled the following inclusion criteria: meeting the El Escorial criteria for definite and probable ALS, being men or nonpregnant women aged 25 to 75 years, and having a progressive course, from 31 to 46 score in the ALSFRS-R scale of disease severity during the preceding inclusion in the trial. After lead-in period for 3 months, autologous MSCs were isolated from bone marrow, expanded in vitro and suspended in about 1 mL autologous cerebral spinal fluid(CSF), 7 patients received an intrathecal MSCs(1 X 10^6) injection twice at an interval of 1 month via a standard lumbar puncture. During 12 months after

MSCs injection, all clinical and laboratory findings were collected for primary outcome measure for safety and disease status of patients were analyzed with ALSFRS-R for secondary outcome measure. No significant major adverse events were reported during trials. MSCs injection was well tolerable except for occurrences of transient headache, myalgia, and back pain. The mean ALSFRS-R score remained stable during the first 6 months of observation, compared to lead-in period(-0.14 Vs. 4.15, $p<0.01$). Moreover, the slope of decline of ALSFRS-R was significantly lower during the first 6 months, and 12 months follow-up period, compared to the lead-in period(-0.023 Vs.1.38, $p<0.01$, 0.262 Vs. 1.38, $p<0.05$) Intrathecal autologous bone marrow derived MSCs therapy is safe and feasible in patients of ALS. Short-term follow-up of ALSFRS-R scores suggests a trend towards stabilization and slow progression of disease. However, the benefit needs to be confirmed in the following large scaled study and long-term follow-up period.

T-3013

ANGIOGENIC PROPERTIES OF SECRETOME FROM ADIPOSE TISSUE DERIVED MESENCHYMAL STROMAL/STEM CELLS (ADSC) IN CARDIOVASCULAR DISEASES AND DIABETES MELLITUS TYPE 2.

Nina Dzhoyashvili
Lomonosov Moscow State University, Moscow, Russian Federation

Cardiovascular diseases (CVD) are the largest reason of global mortality. Among many factors diabetes is a significant contributor to CVD risk. Many animal studies have indicated regenerative potential of mesenchymal stem cells (MSC). MSC are considered the promising therapeutic agents for cell therapy. However, the regenerative potential of MSC can be deteriorated in age-related diseases such as coronary artery disease and diabetes. This knowledge is necessary to provide new insights into molecular and cellular mechanisms underlying many pathological conditions. In addition, these findings will allow us to increase therapeutic potentials of stem/progenitor cells.
We investigate angiogenic properties of adipose tissue derived mesenchymal stromal/stem cells (ADSC) from patients with ischemic heart disease (IHD), diabetes mellitus type 2 (DM2) and heart failure (HF). ADSC were isolated from subcutaneous adipose tissue of patients with IHD (n=23); IHD and DM2 (n=20); IHD and HF (n=12); IHD, DM2 and HF (n=6) and control donors (n=15). ADSC immunophenotype, proliferative and differentiation capacity were similar for all groups. Total tube length formed by EA.Hy926 cells on Matrigel was lower in IHD +DM2 (p=0, 01) and IHD+HF (p=0,029). There was a decreasing trend in IHD group (p=0,101). There wasn't increase in VEGF and PlGF secretion in IHD +DM2 and IHD+HF groups. ENDS and TbS-1 mRNA levels were significantly increased in all groups of patients compared to the control group. There wasn't increase in the secretion of these factors. It may be due to a low stability of these proteins. We showed negative correlations between the total length tube of endothelial cell EA.hy 926 and gene expression of ENDS and TbS-1 in ADSC from IHD+HF and IHD groups. Therefore, we presume that decrease in angiogenic potentials of ADSC from patients with IHD complicated by diabetes and heart failure is due to insufficient pro-angiogenic and more potent anti-angiogenic effects of ADSC secretome.

T-3014

SYNTHETIC PEPTIDE COATED SURFACE FOR CULTURE OF HUMAN MESENCHYMAL STEM CELLS IN DEFINED AND XENO-FREE MEDIA

Katie Slater[1], Jeff Partridge[1], Kerry Thompson[1], Paula Flaherty[1], Susan Qian[1], Greta Minonzio[2], Mauro Gola[2], Gianni Soldati[2], **Deepa Saxena**[1]
[1]*Corning Life Sciences, Bedford, MA, United States,* [2]*Swiss Stem Cell Foundation, Lugano, Switzerland*

Mesenchymal stem cells (MSCs) are multipotent stem cells with the ability to differentiate into bone, cartilage and fat cells. MSCs are an important tool in regenerative medicine; the therapeutic potential of these cells is being evaluated for several disorders. *Ex vivo* expansion of MSCs requires either bovine serum containing media or coating of the culture vessel with human or animal-derived extracellular matrix (ECM) protein. Growing concerns about introducing human and animal-derived pathogens into the culture necessitate the need for an animal free (defined as xeno-free and human origin components-free) culture environment. Also, matrices of biological origin can be

undefined, triggering expensive adventitious agent screening. Self-coating requires additional time, and results in coated vessels with limited shelf-life. Here, we report PureCoat ECM Mimetic Fibronectin peptide and Collagen I peptide, synthetic peptide coated surfaces for culture of cell types that require Fibronectin or Collagen I coating. The peptide surface is pre-coated, synthetic, xeno-free, human-origin components free, and room temperature stable.
In this study human bone marrow derived mesenchymal stem cells were cultured on Fibronectin peptide in Mesencult, and StemPro serum-free, defined and xeno-free media. MSCs exhibited growth and morphology comparable to cells grown on an ECM coated surface. Cells remained multipotent and were successfully differentiated into osteocytes and adipocytes after multiple passages. Immunophenotyping by flow cytometry revealed that the MSC population was positive for CD90, CD105, and CD73 markers. Cells did not express CD34, CD11b, CD19, CD45 and HLA-DR. In another study, adult human adipose tissue- derived mesenchymal stromal cells were cultured on Collagen I mimetic surface in a serum-free medium (SF®). Post-expansion, cells continued to express CD73, CD90 and CD105 markers as determined by flow cytometry, and successfully differentiated into adipocytes and osteocytes. PureCoat ECM Mimetic Fibronectin peptide and Collagen peptide- coated surfaces provide a ready to use alternative to these ECM coatings for culture of cells with clinical potential.

T-3015

TREATMENT OF 23 CASES OF ASEPTIC NECROSIS OF FEMORAL HEAD IN DOGS WITH IMPLANTATION OF AUTOLOGOUS BONE MARROW -DERIVED CELLS AS A MINIMAL INVASIVE THERAPY : A SEVEN YEARS EXPERIENCE.

ANTONIO CROVACE[1], Francesco Staffieri[1], luca lacitignola[1], Giacomo Rossi[2], Edda Francioso[1]
[1]D.E.O.T., UNIVERSITY OF BARI, VALENZANO, Italy, [2]VETERINARY MEDICINE, UNIVERSITY OF CAMERINO, MATELICA, Italy

Objective.Aseptic osteonecrosis of the femoral head or Legg- Calvé- Perthès disease (LCP'D) is a disease that affects young dogs of small breeds and can be considered superimposable to the one that occurs in the children.
In the present study are reported the results of the use of autologous Bone Marrow Mononuclear Cells (BMMCs) and cultured Bone Marrow Stromal Cells (cBMSCs) as a minimal invasive therapeutic treatment of osteonecrosis of the femoral head in dogs.
Material and methods
23 dogs of small size and different breed, 12 males and 11 females, aged from 8 to 15 months affected by monolateral LCP'D were enrolled in this study. The grade of the radiographic features of the disease was estimated according to the Ljunggren 's scale. In 19 dogs the BMMCs have been administered while in the other four the cBMSCs ones, after they were cultured for 3-4 weeks. The bone marrow was collected from each subject from the iliac crest and the mononuclear fraction was separated by a gradient centrifugation. The mean number of BMMCs was of 8.9 x 108±3.9 x 108while the mean number of the cBMSCs was of 8.4x 106± 3.5 x 106. For the BMMCs the ColonyForming Unit (CFU) were evaluated and the mean number was of 5.5 x102 ±5.2 x 102/ml. The cells were suspended in fibrin glue just before the administration and then implanted by transcutaneous injection, under CT or RX guide, using a Jamshidi needle inserted through the femoral head and neck starting at the basis of the trochanter major.
Results
In 21 of the treated dogs the disappearance of pain was observed starting from 3-4 weeks after the cells administration and also a gradual weight bearing on the affected limb up to a complete remission of the symptomatology. In the other 3 cases at ten weeks from the treatment a femoral head and neck ostectomy was performed because the recovery proceeded too slowly and the owners preferred to don't wait anymore. Histological and immunohistochemistry studies were performed on these samples and had evidenced the presence of cartilage and subcondral bone of new formation in the area in which the cells have been implanted.
Conclusions
As to the results obtained, the cell therapy seems to be an effective and minimal invasive therapeutic treatment of the LCP'D . The efficacy could be due to the osteogenetic and anti-inflammatory capacity of the stromal cells which could lead first to the disappearance of the pain and then to a more intense reparative activity with a more precocious sclerosis of the femoral head.

T-3016

MICRORNA 125B INHIBITS HYPOXIA-INDUCED APOPTOSIS OF RAT MESENCHYMAL STEM CELLS BY COTARGETING BAK 1 AND CASPASE 2

Jiyun Lee[1], Byeong-Wook Song[1], Min-Ji Cha[1], Onju Ham[1], Se-Yeon Lee[1], Chang Youn Lee[2], Jun-Hee Park[2], Hyang-Hee Seo[1], Minji Seung[1], Eunhyun Choi[3], Eunmi Choi[4], Il-Kwon Kim[5], **Ki-Chul Hwang**[4]
[1]Yonsei University College of Medicine, Brain Korea 21 Project for Medical Science, Seoul, Korea, Republic of, [2]Graduate School, Yonsei University, Department of Integrated Omics for Biomedical Sciences, Seoul, Korea, Republic of, [3]Yonsei University Health System, Severance Integrative Research Institute for Cerebral & Cardiovascular Disease, Seoul, Korea, Republic of, [4]Yonsei University College of Medicine, Severance Biomedical Science Institute, Seoul, Korea, Republic of, [5]Yonsei University College of Medicine, Cardiovascular Research Institute, Seoul, Korea, Republic of

Adult mesenchymal stem cells (MSCs) are one of popular sources for treatment of cardiovascular disease such as hypertension and ischemic heart disease. However, major obstacle to MSC-based therapy is low viability of transplanted MSCs due to low incorporation between MSCs and resident cardiomyocytes. Recently, microRNAs (miRs) can act as anti- and pro-apoptotic miRs, depending on the cellular events and on the various target genes. In the present study, we hypothesized that miR-125b increases the survival of MSCs in hypoxic condition. To select appropriate miR that is possible to inhibit apoptosis in hypoxic condition, we transfected 14 candidate miRs. Among them, miR-125b showed that the survival of MSCs significantly increased by cell count assay and MTT assay. Also, hypoxic MSCs showed down-regulated expression of miR-125b. We anticipated direct targets of miR-125b, Bak-1 and caspase-2 which are related to apoptotic signaling via the involvement of mitochondria. Using western blotting and luciferase reporter assay, we confirmed that both Bak-1 and caspase-2 are direct targets of miR-125b. Bak triggers mitochondrial outer membrane permeabilization (MOMP) and capsase-2 is required for MOMP as well as the release of cytochrome *c* as an apoptotic factor. Therefore, we confirmed MOMP-related proteins in MSCs transfected with miR-125b under hypoxic condition. The MOMP-related apoptotic proteins, Bcl-2, cytochrome *c*, caspase-3 and caspase-9, were significantly regulated by cotargeting Bak-1 and caspase-2. These results suggest that miR-125b enhances the survival of MSC in hypoxic condition and inhibits MOMP by co-targeting Bak1 and caspase2.

T-3017

MICRORNA 126 MODULATES RAT MESENCHYMAL STEM CELLS SURVIVAL BY INHIBITION OF TRAF7 VIA REDUCTION OF THE TNF ALPHA-INDUCED JNK ACTIVATION

Jun-Hee Park[1], Byeong-Wook Song[2], Min-ji Cha[2], Onju Ham[2], Se-Yeon Lee[2], Chang Youn Lee[1], Jiyun Lee[2], Hyang-Hee Seo[2], Minji Seung[2], Eunhyun Choi[3], Eunmi Choi[4], Il-Kwon Kim[5], **Ki-Chul Hwang**[4]
[1]Graduate School, Yonsei University, Department of Integrated Omics for Biomedical Sciences, Seoul, Korea, Republic of, [2]Yonsei University College of Medicine, Brain Korea 21 Project for Medical Science, Seoul, Korea, Republic of, [3]Yonsei University Health System, Severance Integrative Research Institute for Cerebral & Cardiovascular Disease, Seoul, Korea, Republic of, [4]Yonsei University College of Medicine, Severance Biomedical Science Institute, Seoul, Korea, Republic of, [5]Yonsei University College of Medicine, Cardiovascular Research Institute, Seoul, Korea, Republic of

Mesenchymal stem cells (MSCs) have tremendous prospects for regenerative medicine and have been studied for the treatment of heart failure. However, the efficiency of MSCs is hindered by low survival after injection of cells in post-infarct heart. To overcome the defects, many researchers use microRNAs (miRs) which may have anti-apoptotic functions in MSCs. MiRs are recently associated with various functions such as the modulation of cellular differentiation, proliferation, apoptosis, and so on. In this study, we hypothesized that miR-126 protects MSCs

from apoptosis induced by hypoxic condition targeting TNF receptor-associated factor 7 (TRAF7). We transfected 36 miRs related to cell survival and proliferation into MSCs. Among them, miR-126-overexpressed MSCs significantly increased survival rate compared to normal and other miR-transfected MSCs. Also, we found that one of predicted targets is TRAF7, which is essential for activation JNK and regulates the degradation of an anti-apoptotic protein, cellular FLICE-inhibitory protein (c-FLIP). The protein level of TRAF7 was interestingly decreased in miR-126-transfected MSCs. c-FLIP is known to be catalytically inactive caspase-8 and -10 to protect from apoptosis mediated by death receptors. The expression levels of apoptosis signaling-related proteins and downstreams of JNK pathway, phosphorylate-JNK, c-FLIP, caspase-8/-10, and Bcl-2, were meaningfully regulated in MSC transfected with miR-126. Collectively, our results indicate that miR-126 is critical in enhancement of MSC survival in TNF-alpha-induced harsh condition via targeting TRAF7.

T-3018

MESENCHYMAL STEM CELLS-DERIVED MICROVESICLES EXHIBIT A RENOPROTECTIVE EFFECT BY MIRNA-MEDIATED EPIGENETIC REPROGRAMMING OF TUBULAR EPITHELIAL CELLS

Danilo C. Almeida
Medicine, Federal University of São Paulo, São Paulo, Brazil

Mesenchymal stem cells-derived microvesicles exhibit a renoprotective effect by miRNA-mediated epigenetic reprogramming of tubular epithelial cells

Currently, acute kidney injury (AKI) is great health problem in the world with limited treatments options. In this sense, the use of mesenchymal stem cells (MSC) is an innovative and affordable way to treat several nephropathies. MSCs have powerful immunotherapeutic potential besides presenting as effector agents in the epigenetic regulation of gene expression by microvesicles secretion containing miRNAs. Therefore, this study aimed to assess the global expression profile of miRNAs as well as the molecular and cellular mechanisms involved in MSC-mediated renoprotection using an experimental model of nephropathy. Therapeutically, the MSCs treatment promoted a clinical and functional improvement in transplanted animals showing healthy clinical score, decreased levels of creatinine and urea (renal fuctional markers), reduction in the rate of blood lactate and a lower index of proteinuria. Moreover, we verified after treatment with MSCs, an increase in the proliferation index concomitant with significant reduction of apoptosis score on renal tissues. It was also observed a modulation of the cytokine profile (serum and tissue) to an anti-inflammatory profile after treatment with MSCs. In addition, we found that both local and systemic immunoregulation are directly associated with specific inhibition of the NFkB pathway in tubular cells via modulation of signaling proteins (IKK-α, IkBα, NFkB-p65), cell receptors (TNFR, TLR-2 and TLR-4) and pro-inflammatory molecules (C3, Csf3, Egr-1, IL-6, IFNg, IL-1β and TNF-α). In addition, we also studied the modulation profile of miRNAs in renal tissues during therapy with MSCs. Around 528 miRNAs were analyzed, which 464 were similarly expressed, 61 were up-regulated and 3 were down-regulated with special participation of miR-377 and miR-141. Complementary, the proteins involved in the biosynthesis of miRNAs (Dicer 1, Drosha, Argonaut 2) were up-regulated in injury and down-modulated after MSC injection. In order to evaluate the mechanism involved, we conducted several experiments *in vivo* and *in vitro* using MSC and their microvesicles derived (MVs-MSC). Both MSCs and MVs-MSC, exerted a local renoprotective effect with low levels of serum creatinine and urea, decreased tubular apoptosis (Caspase-3, Tunel, Bad, Bax and Bcl-2), less oxidative stress (NO and H2O2) and reduction of pro-inflammatory cytokines levels (TNF-α, IL-17 and IFN-γ). Furthermore, the treatments significantly increases the level of anti-inflammatory molecules (IL-10 and IL-4) and renoprotective genes (VEGF, HGF, IGF and HO-1) beyond reduced the mitochondrial oxidative stress in tubular cells *in vitro*. The pre-treatment with RNAses, annulled the effect of MVs-MSC and the use of DNases and proteases did not change the phenotype. In conclusion, this work demonstrates that the mechanisms behind of MSC-associated renoprotection could involve epigenetic reprogramming of tubular cells via miRNA modulation. In the future, we hope to characterize the precise mechanism related with MSC regenerative process, as well as identify potential miRNAs biomarkers of injury or tissue regeneration, facilitating their translation into clinical practice.

T-3022

ENHACING THE THERAPEUTIC POTENTIAL OF MESENCHYMAL STEM CELLS FOR THE TREATMENT OF CENTRAL NERVOUS SYSTEM AUTOIMMUNITY

Natalie Payne[1], Guizhi Sun[1], Martin Short[1], Courtney McDonald[2], Christopher Siatskas[3], Daniella Herszfeld[1], Claude CA Bernard[1]

[A]Australian Regenerative Medicine Institute, [B]Monash Institute of Medical Research, [C]Department of Anatomy and Developmental Biology, [1]Monash University, Clayton, Victoria, Australia

Mesenchymal stem cells (MSCs) are considered to hold great potential in regenerative medicine owing to their ease of isolation from most postnatal tissues, broad immunomodulatory and reparative properties as well as their capacity to home sites of inflammation and injury. The beneficial effects of MSCs demonstrated in pre-clinical animal models is believed to occur predominantly through the secretion of soluble factors that act shortly after MSC delivery. Modulation of the MSC secretome, using pre-conditioning strategies such as exposure to cytokines or hypoxia, upregulates a number of factors that may improve MSC survival and function in vivo. Alternatively, MSCs can be engineered to produce supraphysiological levels of therapeutic proteins such as growth factors or anti-inflammatory cytokines that further enhance their efficacy.
Multiple sclerosis (MS) is an autoimmune disease targeting the central nervous system (CNS) that is characterized by multifocal inflammatory and demyelinating lesions and axonal degeneration. In the current study, we characterized the immunomodulatory properties and homing potential of tissue-specific MSCs in vitro, their ability to suppress an MS-like disease in mice termed experimental autoimmune encephalomyelitis (EAE), and investigated pre-condioning methods to enhance their therapeutic effect. MSCs derived bone marrow (BM), adipose tissue (Ad) and umbilical cord Wharton's jelly varied in their homing potential, with Ad-MSCs expressing a broader repertoire of chemokine receptor mRNA and cell surface adhesion molecules. In contrast, BM-MSCs were more efficient at inhibiting in vitro T-cell responses. In vivo, Ad-MSCs migrated to inflammatory sites in the periphy and CNS, and suppressed disease in both chronic progressive and relapsing-remitting EAE models. Pre-conditioning with cytokines relevant to MS was found to modulate the MSC secretome, enhancing mRNA expression of a number of neurotrophic growth factors. Using a lentiviral vector-based approach, Ad-MSCs were engineered to overexpress the anti-inflammatory cytokine interleukin-10. The efficacy of gene modified Ad-MSCs delivered to EAE mice at the time of T-cell priming was enhanced due to the inhibition of antigen presenting cell function and Th17-mediated neuroinflammation. Collectively our results show that pre-conditioning of MSCs through cytokine exposure or genetic modification can enhance their potential for the treatment of autoimmune disease targeting the CNS.

T-3023

A NOVEL SYSTEM FOR SIGNIFICANT EXPANSION AND EPIGENETIC NEURAL INDUCTION OF HUMAN MESENCHYMAL STEM CELLS.

Andrea L. Marty[1], Kyle E. Stehlik[2], Laura A. Hibbard[1], Arshak R. Alexanian[2], Bradley H. Garcia[1]

[1]Primorigen Biosciences Inc., Madison, WI, United States, [2]Department of Neurosurgery, VA Medical Center, Milwaukee, WI, United States

Current mesenchymal stem cell-based therapies typically require rapid harvest, purification, expansion, and manipulation of cells to attain therapeutically relevant numbers of cells with the desired phenotype. For acute spinal cord injury (SCI), one promising approach requires effective neural induction of expanded MSCs prior to transplantation to restore locomotion, as measured by the Basso, Beattie, and Bresnahan (BBB) scale in SCI animal models. Thus, technologies that improve MSC expansion and/ or neural induction are highly relevant to successful MSC based cellular therapies for SCI. Our previously reported epigenetic-based neural induction strategy [1] confirmed that NI-hMSCs partially differentiated in limited numbers from low passage, conventionally cultured hMSCs reduced cavity volume, promoted white matter sparing, and significantly improved locomotor recovery in rat models of SCI. We now report a new method that enables significantly more rapid MSC expansion and thorough neural induction by using human platelet lysate and macromolecular crowding (MMC) to greatly enhance our epigenetic neural induc-

tion method [1]. Various combinations of the platelet lysate and MMCs increased the proliferative rate of MSCs by over 500% and improved the expression of numerous important markers of neural precursors above the published control, including BDNF (167%), GDNF (115%), NCAM (1425%) and MAP2 (1324%). The system also improved MSC differentiation into adipocytes (875%) and osteocytes (512%). In vivo transplant experiments for acute SCI are underway to establish that BBB scores resulting from these potent NI-hMSCs are at least equivalent to our previously reported strategy. Results will be presented here.

T-3024

HUMAN UMBILICAL CORD BLOOD MESENCHYMAL STEM CELLS ALLEVIATE EXPERIMENTAL COLITIS IN MICE VIA NOD2-COX2 AXIS ACTIVATION

Hyung-Sik Kim, Tae-Hoon Shin, Byung-Chul Lee, Kyung-Rok Yu, Yoojin Seo, In-Sun Hong, Soon Won Choi, Kwang-Won Seo, Jong-Hwan Park, Kyung-Sun Kang
Seoul National Univ, Seoul, Korea, Republic of

Human umbilical cord blood mesenchymal stem cells alleviate experimental colitis in mice via NOD2-COX2 axis activation

Hyung-Sik Kim,[1*] Tae-Hoon Shin,[1] Byung-Chul Lee,[1] Kyung-Rok Yu,[1] Yoojin Seo,[1] Min-Soo Seo,[1] In-Sun Hong, [1] Soon Won Choi[1], Kwang-Won Seo[1], Gabriel Núñez,[2] Jong-Hwan Park,[3†] and Kyung-Sun Kang[1†]

[1]Adult Stem Cell Research Center, College of Veterinary Medicine, Seoul National University, Seoul 151-742, South Korea

[2]Department of Pathology and Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, MI 48109

[3]Department of Biochemistry, College of Medicine, Konyang University, Daejeon 302-718, South Korea
* Presenter, lio2913@naver.com, +82-10-8533-3922, Ph.D. candidate
Running title: Role of Nod2 on adult stem cells
[†]Correspondence:
Kyung-Sun Kang, D.V.M., Ph. D
Adult Stem Cell Research Center, College of Veterinary Medicine, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-742, South Korea
Tel. +82-2-880-1246
E-mail: kangpub@snu.ac.kr
Jong-Hwan Park, D.V.M., Ph. D
Department of Biochemistry, College of Medicine, Konyang University,
28 Wonang-1-ro, Gwanjeo-dong, Seo-gu, Daejeon 302-718, South Korea
Tel. +82-42-600-6455
E-mail: jonpark@konyang.ac.kr
Category: Immunology and Stem Cells
Pre-clinical and Clinical Applications of Mesenchymal Cells
Abstract
Background & Aims: Nucleotide-binding oligomerization domain 2 (NOD2) is known to be associated with the development of Crohn's disease. Although studies report that NOD2 modulate the host intestinal inflammation, little is known about the role of NOD2 on the transplanted cell function. In this study, we investigated whether NOD2 has any role on the anti-inflammatory function of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) for transplantation. **Methods:** Dextran sulfate sodium (DSS)- or trinitrobenzene sulfonic acid (TNBS)-induced colitic mice were infused with NOD2-activated or –inhibited hUCB-MSCs and mice were evaluated for cytokines production and the recruitment of leukocytes. To determine the inhibitory property of hUCB-MSCs on the proliferation of human mononuclear cells (hMNCs), BrdU assay was performed. **Results:** Systemic infusion of hUCB-MSCs protected mice from lethality and reduced the severity of colitis. These properties of hUCB-MSCs were markedly enhanced by NOD2 activation with its ligand, muramyl dipeptide (MDP). Administration of NOD2-activated hUCB-

MSCs increased anti-inflammatory responses such as colonic IL-10 production and Foxp3+ cells infiltration and suppressed pro-inflammatory cytokines production. The proliferation of hMNCs was significantly inhibited by the presence of culture media from hUCB-MSCs stimulated with MDP. MDP induced prolonged PGE_2 production in hUCB-MSCs via the NOD2-RIP2 pathway, which was responsible for the suppression of hUCB-MNCs proliferation. PGE_2 produced by hUCB-MSCs in response to MDP increased IL-10 production and regulatory T-cell population. **Conclusions:** NOD2 activation promotes the protective effect of hUCB-MSCs against colitis and augmented their ability to suppress hMNCs proliferation by inducing PGE_2 production.

Keywords: Mesenchymal stem cells; NOD2; immunomodulation; colitis

T-3025

AMNIOTIC FLUID DERIVED MESENCHYMAL STROMAL CELLS: POTENTIALLY HAZARDOUS IN THE TREATMENT OF TRAUMATIC BRAIN INJURY.

Robert A. Hetz, Fabio Triolo, Scott D. Olson, Supinder Bedi, Daniel J. Kota, James C. Roye, Philippa Smith, Mary-Claire Day, Bryan Dicarlo, Charles S. Cox
Pediatric Surgery, University of Texas Health Science Center Houston, Houston, TX, United States

Indroduction: Mesenchymal stromal cells (MSCs) have shown promise as an affective treatment in numerous inflammatory diseases. Preclinical data from our lab and others using bone marrow derived MSCs and multipotent adult progenitor cells (MAPCs) for the treatment of traumatic brain injury (TBI) have demonstrated significant improvements. Specifically, there is a decrease in blood brain barrier (BBB) permeability (surrogate for cerebral edema), and long term cognitive improvements (spatial learning). We have isolated and expanded clinical grade amniotic fluid (AF) derived MSCs (AFMSC) as a potential alternative source for therapeutic progenitor cells. AF is an attractive cell source as amniocentesis is relatively easily performed with little risk to the mother/fetus. These cells can go on to be banked for use at any point in the infant's/patients life for the treatment of numerous diseases. **Methods:** A rodent control cortical injury (CCI) TBI model was used (depth of 3.1 mm, rate of 5.6 m/s, dwell time of 200 msec). AFMSC treatment was administered intravenously at 2 and 24 hours after injury. There were three groups of experimental animals: sham (n=4), CCI-Alone (n=8), and CCI-AFMSC (n=6) (treatment dose of $1x10^6$ cells/kg). BBB permeability was assessed by measuring Evans blue dye extravasation via plate based flurometric analysis. AFMSCs were further tested *in vitro* for their ability to suppress TNF-alpha production. Co-cultures of AFMSCs with mouse spenocytes were then activated with LPS (1ug/ml) to stimulate TNF-alpha production. Three splenocyte to AFMSC ratios were tested, 1:5, 1:20, and 1:400 with bone marrow (BM) MSCs in the same ratios as a positive control. TNF-alpha production was evaluated using plate based ELISA. **Results:** CCI alone rats showed a significant increase in BBB permeability compared to sham injured animals (0.54 +0.036 to 0.09 +0.008, p=0.001). AFMSC treated rats showed a significant increase in BBB permeability compared to CCI alone rats (0.82 +0.12 to 0.54 +0.036, $p<0.05$). TNF inhibition of activated splenocytes co-cultured with AFMCS show no significant decrease in TNF production compared to control LPS activated splenocytes at any ratio. At a ratio of 1:20 splenoctyes to AFMSC there was a significant increase in TNF production when compared to the control (397.7 +15.72 to 344.1 +4.99, p=0.016). BMMSCs co-cultured with splenocytes showed significant decrease in TNF production at all ratios. **Conclusion:** Based on our results, not all MSCs should be considered equal in the treatment of inflammatory disease, and specifically treatment with AFMSCs should be preceded with caution. We have demonstrated in our lab that AFMSCs have the capability of being proinflammatory in an *in-vitro* model of inflammation and are able to corroborate this with *in-vivo* data confirming worsening of the inflammatory response in a rat TBI model based on worsening of BBB permeability.

T-3026

INTRASPLENIC TRANSPLANTATION OF ALLOGENEIC MESENCHYMAL STROMAL CELLS REVERTS HYPERGLYCEMIA IN TYPE 1 DIABETIC MICE

Juliana N.U Yaochite[1], Carolina Caliári-Oliveira[1], Lourenço S. Neto[2], Kelen C.R Malmegrin[2], Dimas T. Covas[2], Júlio C. Voltarelli[2], Eduardo A. Donadi[1]

[A]Basic and Applied Immunology, [1]School of Medicine of Ribeirão Preto, University of Sao Paulo, Ribeirão Preto, Brazil

Introduction: The transplantation of mesenchymal stromal cells (MSCs) has been proposed to be one possible therapeutic alternative in type 1 diabetes. However, one challenge associated with MSCs transplantation is the route of administration for efficient cell delivery. The intravenous route has been widely used but the entrapment of the MSCs inside the lungs is a recurrent problem that should be avoided. Considering this scenery, new strategies of cell delivery are needed to improve cell migration into the damaged tissue. **Aim:** Evaluating the therapeutic potential of intrasplenic transplantation of allogeneic MSCs in experimental type 1 diabetes. **Methods:** Diabetes was induced in C57BL/6 male mice by intraperitoneal injection of streptozotocin (40 mg/Kg) for 5 consecutive days. Mice whose non fasting blood glucose levels were over 250 mg/dL on two consecutive measurements were considered diabetic. Allogeneic MSCs were isolated from adipose tissue of Balb/c male mice. For the treatment of diabetic mice, 1 x10^6 MSCs were administrated by intrasplenic injection in hyperglycemic mice (n=10) twenty days after diabetes induction. At the same time, a control group of diabetic mice (n = 6) was intrasplenically injected with PBS. The blood glucose levels were monitored periodically (Accu-Check Active, Roche). The glucose tolerance test (GTT) was performed 30 days after MSCs/PBS injection. The glucose (1.5 mg/g body weight) was administrated intraperitoneally in 10 hours fasting mice, and blood glucose levels were determined before and 15, 30, 60, 120 and 180 minutes after glucose administration. Glycosuria was also evaluated at day 30th post-treatment using Diabur Test 5000 strips (Roche). **Results:** We observed that the intrasplenic administration of allogeneic MSCs in diabetic C57BL/6 mice could recover the hyperglycemia in 70% of diabetic animals. Looking at the responders MSCs-treated mice, the mean of blood glucose levels before the MSCs injection was 323.8 + 32.6 mg/dL and twenty days after MSCs treatment the levels of glucose decreased to 175.57 + 40.3 mg/dL ($p<0.0001$). Thirty days after MSCs transplantation, the fasting glycemia of MSCs-treated group was significant lower when compared with the control group (165.55 + 39.9 mg/dL *versus* 260.83 + 77.3 mg/dL, p=0.007). During GTT, the treated group showed a significant improvement of the peripheral response to glucose in all time points evaluated after glucose administration. The area under the curve of MSCs-treated group was statistically significant lower when compared with the untreated group (42.433 *versus* 71.993; p=0.009). Moreover, the glycosuria levels were significantly lower in the MSCs-treated mice (0.56 g/dL) when compared with the control mice (3.4 g/dL; $p<0.05$). Thirty five days after MSCs transplantation, the mean
of non-fasting blood glycemia of MSCs-treated group was lower than in the control group (198.5 mg/dL *versus* 339.66 mg/dL; p=0.0003). **Conclusion:** The intrasplenic transplantation of allogeneic MSCs was effective in reverting hyperglycemia, improving glucose control in diabetic mice. The intrasplenic route of MSCs injection represents a new approach to avoid MSCs pulmonary entrapment with promising results in type 1 diabetes setting.

T-3027

EFFICIENT EXPANSION OF HUMAN MESENCHYMAL STEM CELLS ON SYNTHETIC MICROCARRIERS IN SERUM-FREE, DEFINED MEDIUM USING BEAD-TO-BEAD TRANSFER.

Jennifer Weber[1], Martial Hervy[2], Yue (Jerry) Zhou[1], David Henry[2], Paul Gagnon[3], Paula Dolley-Sonneville[1], Zara Melkoumian[1]

[1]Corning Incorporated, Corning, NY, United States, [2]Corning Incorporated, Corning SAS, Avon, France, [3]Corning Incorporated, Kennebunk, ME, United States

Cell-based therapies require efficient production of a consistent cell product in large quantities. Microcarriers provide a three-dimensional culture environment that enables rapid cell expansion in dynamic conditions. Traditional microcarriers generally require time-consuming and labor-intensive preparation that can affect the consistency and efficiency of cell production. Further, serum-containing medium may be required to facilitate cell attach-

ment and expansion in dynamic conditions. These limitations hinder the use of microcarriers for cellular therapeutics and biopharmaceutical manufacturing.
Here we report new synthetic, ready-to-use microcarriers for scalable cell production in dynamic conditions. These microcarriers were used to expand human bone marrow-derived mesenchymal stem cells (hMSCs) for multiple passages in serum-free, defined medium (stemgro hMSC medium). We demonstrate cell expansion by bead-to-bead transfer without the need for enzymatic dissociation. Cells retained typical spindle-like morphology, cell surface marker expression profile, multipotency, and normal karyotype. Further, we demonstrate short-term expansion of hMSCs on synthetic microcarriers in several other commercially-available media.

T-3028

STERILE, FREEZE-DRIED MICROVASCULAR TISSUE RETAINS MANY BENEFITS OF STEM CELLS

Dale Peterson[1], Ralph-Heiko Mattern[1], Mohammad El-Kalay[2], Dirk Hunt[3], Mary Pat Moyer[3]
[1]Tornier, Inc., San Diego, CA, United States, [2]Regenerative Medicine Consultant, San Diego, CA, United States, [3]INCELL Corp., San Antonio, TX, United States

Introduction
Various stem cell preparations have shown beneficial effects in animal and clinical studies for many indications despite the poor survival of the administered cells. This observation spurred the discovery of new mechanisms of action for stem cells besides the early concept that the cells engraft and differentiate into desirable tissues. The new mechanisms include enhancing angiogenesis, diminishing inflammation and apoptosis, and recruiting host cells. These findings led to the hypothesis that stem cells do not need to be viable to produce most of the benefits seen in vivo. Use of nonviable stem cells alleviates safety concerns that have hindered adoption such as fears of cancer stem cells, aberrant differentiation, and formation of inappropriate tissues. This concept was tested using microvascular tissue, a rich source of stem and progenitor cells.
Objective
Prepare sterile, lyophilized microvascular tissue and assess its bioactivity.
Methods
Microvascular tissue was isolated from human cadaveric adipose tissue by mincing followed by enzyme digestion and centrifugation to remove fat. The microvascular tissue was resuspended in cryopreservation buffer (1:1 mix of M3:DC and EZ-CPZ, INCELL), dispensed into vials, lyophilized and radiation sterilized.
The resulting processed microvascular tissue was assayed for cell counts, viability, phenotype, CFU-F, and bioactivity. Cells were counted using a hemacytometer after staining with DAPI, to identify nucleated cells, and Trypan blue. Phenotyping was performed with an EVOS fluorescence microscope and image analysis using antibodies against collagen type IV, CD34, CD44 and CD45. CFU-F assays were performed using an enriched stem cell culture medium (M3:10). An endothelial cell migration assay was run in Transwell plates with early passage HUVEC and counted at 48 hours.
Results
The surgically resected adipose tissue yielded 0.72 - 0.88 x 106 cells/gm adipose tissue. 80-90% of the cells were intact following digestion, 15-28% following lyophilization, and 1-4% following radiation sterilization. No colonies grew after lyophilization or sterilization in CFU-F assays.
Phenotyping results were unchanged for the microvascular tissue following digestion, lyophilization and sterilization. The tissue expressed CD34 (>60%), CD44 (>75%), CD45 (<10%), and collagen type IV (>60%).
The lyophilized tissue before and after radiation sterilization showed equivalent stimulation of HUVEC migration, ranging from 82 to 126% of the level of the EGF-spiked positive control.
Conclusions
These data indicate cells and proteins were well-preserved in this lyophilized, sterilized microvascular tissue. Surprisingly, the sterilized tissue still presented the same proportion of surface markers and showed the same level of activity in the cell migration assay. These results suggest the possibility of a stable, sterile, off-the-shelf tissue

product that retains many of the beneficial properties of stem cells. This tissue preparation is now being tested in animal injury models.

T-3031

AN ORGANOTYPIC EPIGENETIC SIGNATURE DISCRIMINATES HUMAN STROMAL PROGENITORS AND CORRESPONDS TO THEIR BONE AND MARROW NICHE-FORMING CAPACITY IN VIVO

Andreas Reinisch[1], Nathalie Etchart[2], Nicole A. Hofmann[2], Anna Ortner[2], Katharina Schallmoser[3], Dirk Strunk[4]
[1]ISCBRM, Stanford University, Stanford, CA, United States, [2]Medical University, Graz, Austria, [3]Paracelsus Medical University, Salzburg, Austria, [B]Experimental and Clinical Cell Therapy Institute, [3]Paracelsus Medical University, Salzburg, Austria

Mesenchymal stem/progenitor cells (MSCs) from various tissues are currently tested in clinical trials despite limited understanding of their in vivo behavior. In this study we used human MSCs from adult and fetal tissue in a recently established ectopic human bone and marrow niche (HuNiche) formation mouse model (Blood 119:4971) to better select the appropriate cell source for therapeutic application. We asked whether MSCs derived from bone marrow (BM), white adipose tissue (WAT) and umbilical cord (UC), compared to skin fibroblasts, bear an equivalent bone and marrow niche formation potential in vivo. To gain mechanistic insight gene expression profiling and DNA methylation analysis on a novel high-resolution 450K-CpG methylation array was employed and results were correlated with in vivo differentiation potential.
Comparative 450K-CpG methylation array analysis revealed a tissue-specific epigenetic signature virtually corresponding to the in vivo differentiation potential. Unsupervised hierarchical clustering showed a similarity between biological replicates within a single organ source. Principal component analysis separated DNA methylation profiles according to the tissue of origin. Comparing BM-derived with the non-BM MSCs we found 4,721 (0.97%) significantly ($p < 0.05$) differentially methylated CpG-sites (52.13% hypermethylated, 47.87% hypomethylated). Among the most significant, >200 CpGs corresponded to genes involved in cartilage and bone formation. DNA-methylation patterns within CpG-sites of selected genes (Runt-related transcription factor 3, RUNX3; osteocalcin, BGLAP) inversely correlated to transcription as confirmed by qRT-PCR and (e.g., for RUNX3) significant differences in protein levels suggesting a direct correlation between gene expression and functional protein production.
MSCs were transplanted subcutaneously in immune-deficient NSG mice (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) to test for their natural bone and marrow niche formation potential. BM-derived MSCs reproducibly developed into mature bone (17/17 donors) through an endochondral ossification process with subsequent marrow niche formation (10/17 donors) in ectopic subcutaneous ossicles as shown by osteosensitive near-infrared imaging, computed tomography and pentachromatic histopathology. The newly formed hematopoietic microenvironments attracted a complete mouse hematopoiesis including immature lineage negative, Sca-1 positive, c-kit positive (LSK) hematopoietic stem/progenitor cells (HSPCs). Non-BM-derived MSCs lacked bone and marrow niche-forming potential and did not attract hematopoietic cells (0/9 donors). Induction of human hematopoietic chimerism by transplantation of umbilical cord blood (UCB)-derived CD34+ HSPCs in advance of subcutaneous ectopic bone and marrow establishment resulted in the immigration of re-transplantable human hematopoiesis into the extra-medullary ossicles.
Thus, MSCs from BM but neither fibroblasts nor MSCs from WAT or UC showed a presumably epigenetically imprinted endochondral bone and marrow formation capacity favoring BM-MSCs for skeletal regeneration. The HuNiche model should also be suited for studying normal and malignant hematopoiesis regulation in an ectopic human marrow with subsequent human hematopoietic engraftment that mimics the clinical BM transplantation reality.

T-3032

MULTIPOTENTIAL STROMAL CELL (MSC) IS A PROMISE MAINSTAY TREATMENT FOR THE RESTORATION OF ABDOMINOPELVIC CHRONIC SEVERE DAMAGES INDUCED BY RADIOTHERAPY WITHOUT SIDE EFFECTS.

Chapel Alain, Sabine Francois, bruno Lhomme, marc bendritter

PRP-HOM/ SRBE, Institute of Radioprotection and Nuclear Safety, fontenay aux roses, France

The majority of radiotherapy treatments concern the pelvic area to treat prostate, bladder, uterus, and rectum cancers. Five years after treatment, 5 to 10 % of patients still develop late complications. Radiotherapy efficacy is governed by an optimal compromise between tumor control and normal tissue damage, i.e the risk/benefit ratio. The Laboratory of Radio pathology and Experimental Therapy (LRTE) is studying biotherapy for the treatment of the deleterious effects of ionizing radiations on normal tissues and cells. Application is the improvement of radiotherapy by reducing the injuries to the adjacent healthy tissues when irradiating tumour cells without inducing side effect. The main objective of this work is to study the possible effects of MSC on residual tumor progression after radiotherapy.
To study the influence of MSC on tumor progression; these stem cells are injected intravenously during the early stages of carcinogenesis induced by intrarectal instillation of an alkylating agent in a rat model.
We demonstrate an inhibitory effect of MSC in colorectal carcinogenesis. We have shown that MSC injection reduced the number of adenocarcinoma and extends the life of animals. The anti-cancer effect of MSC was mediated by their immunologic properties on tumour. In adenocarcinoma of CSM-treated rats compared to rats treated with MNNG alone, monocyte/ macrophage CD68+ infiltration was lower and lymphocytes CD3+ higher. IL-1, TNF protein contents in the tumor stroma of adenocarcinoma were significantly lower. In early stage of cancerogenesis when the first histological modifications could be observed, MSC lowered to normal content of IL6, IL-1 IFN-γ. MSC infusion promotes the inhibition of production of pro-inflammatory cytokines secreted by macrophages. Macrophages seem to turn into regulatory cells involved in phagocytosis. This inhibition results from the suppression of Th1-dependent cytokines IFN-γ and IL12. In the rats colons (MNNG + CSM), the rate of INF-γ, IL1 and IL6 was significantly reduced suggesting decreased activity of NK cells, CD8 + lymphocytes and endothelial cells. The simultaneous decrease of IL-6 and TGF-β suggests a restoration of Th17 cell activity, decreased rTh17 cell activity, and decreased the RegT recruitment. The second step in our study concerns the miRNA expression analysis; this work was performed in comparing groups MNNG versus MNNG + MSC. The first analyzes shows that some miRNAs are highly expressed in the animals receiving MSC infusion (mi-150 and miRNA-7). The mi-150 is described as inhibiting cell proliferation and tumor invasion. The mi-RNA-7 regulates negatively the pathway EGFR / AKT promoting cell death and reducing the risk of hypermethylation. MSC infusion during the early stages of colon carcinogenesis modulates the expression of several miRNAs that are likely to contribute to an antitumor effect.
MSC infusion appears to have early and durable action on colon cancer development at early stage; by modulating the immune component of the tumor microenvironment (tumor cells non-tolerant) and mi-RNA expression (decreased proliferation and increased cell death). All these changes induced by the MSC injection have repercussions in the long term by reducing the number of tumors developed. This study is the first step toward use of MSC in therapy to alleviate late severe pelvic radiotherapy damages.

T-3033

TARGETED DELIVERY OF THERAPEUTIC MESENCHYMAL STEM CELLS (MSC) TO KIDNEYS DURING ACUTE TUBULAR NECROSIS BY PULSED FOCUSED ULTRASOUND (PFUS) INCREASES CELLULAR HOMING AND IMPROVES RENAL FUNCTION

Scott R. Burks[1], Pamela A. Tebebi[2], Saejong J. Kim[1], Ali Ziadloo[1], Ben A. Nguyen[1], Victor Frenkel[2], Joseph A. Frank[1]
[1]Radiology and Imaging Sciences, National Institutes of Health, Bethesda, MD, United States, [2]Biomedical Engineering, Catholic University of America, Washington, DC, United States

Introduction: Stem cell therapies are highly promising, but targeted delivery to pathological loci following systemic infusion remains problematic. Circulating stem cells home by tethering to activated endothelium and transmigrating into the parenchyma in response to cytokine/chemokine gradients. Therapeutic stem cells can home to pathological loci during the acute inflammatory response, but often fail to home after it has resolved. We have previously shown pulsed focused ultrasound (pFUS), likely through mechanotransduction, drives a transient local molecular biological response in healthy kidney tissue leading to enhanced homing permeability and retention (EHPR) of MSC to sonicated tissue. MSCs have been shown to have protective/regenerative therapeutic benefits during acute tu-

bular necrosis (ATN). We examined whether pFUS could further increase the number of MSC homing to diseased kidneys, both during and outside of the acute inflammatory period, and whether increasing targeted delivery of MSC to diseased kidneys had additional therapeutic benefit.
Methods: C3H mice were given a single injection of cis-platinum (15 mg/kg) to induce ATN. Kidneys were treated with pFUS either unilaterally or bilaterally (1 MHz, 40 W, 5% duty cycle, 5 Hz repetition frequency) without microbubbles contrast agents either 1 day or 4 days post-ATN. Human MSC were IV injected 2 hr after pFUS. At various times after treatment, mice were euthanized and serum and tissues were harvested for determination of MSC homing, physiological, histological, and molecular analyses.
Results: pFUS to ATN kidneys alone augments the constellation of cytokines, chemokines, and trophic factors and leads to targeted homing of MSC to treated kidneys on both 1 or 4 days post-ATN. These time points reflect differences in the intensity of acute inflammation (lower at Day 1 and higher at Day 4). To examine the therapeutic potential of enhanced homing, other mice were given cis-platinum and then treated with MSC alone, pFUS alone, or pFUS+MSC on Day 1 post-ATN and assessed on Day 5 post-ATN. pFUS+MSC-treated kidneys retained greater numbers of MSC compared to mice receiving MSC alone. Furthermore, targeting additional MSC with pFUS had greater protective effects than MSC alone, while pFUS alone had no effect on the progression of ATN. pFUS+MSC showed less pronounced disease histologically (necrosis and apoptosis) and expressed higher levels of regenerative signaling cues. Most importantly, pFUS+MSC mice showed improved renal function, measured by blood urea nitrogen and serum creatinine, compared to mice that received MSC alone.
Discussion: pFUS is a potentially powerful tool to noninvasively and nondestructively direct stem cell migration in vivo. pFUS drives a complex local biological response that essential creates a “molecular zip code” which can be capitalized for EHPR of therapeutic cells. pFUS therefore provides spatio-temporal control over cell homing processes_it can increase the number of cells to a location and enables targeting of cells at early or late time points during disease when homing by endogenous mechanisms may be minimal. Furthermore, therapeutic efficacy of MSC in this model of ATN was improved by targeting greater numbers of cells to diseased kidneys compared to using untargeted MSC.

T-3034

IN VITRO GENERATION OF A SCAFFOLD-FREE TISSUE-ENGINEERED CONSTRUCT (TEC) DERIVED FROM HUMAN SYNOVIAL MESENCHYMAL STEM CELLS (MSCS) WITH CHEMICALLY DEFINED SERUM-FREE MEDIA, STK1® AND STK2®

Jinchang Shao[1], Shin-ichi Hasegawa[1], Miki Suzuki[1], Yuki Katsura[1], Maiko Hara[1], Masaya Matumoto[1], Norihiko Sugita[2], Yu Moriguchi[2], Kazunori Shimomura[2], Hideki Yoshikawa[2], Koichiro Tsuji[1], Yukio Kato[3], Norimasa Nakamura[2]
[1]Research and Development Division, Two Cells Co., Ltd., Hiroshima, Japan, [2]Orthopedics, Osaka University Graduate School of Medicine, Osaka, Japan, [3]Dental and Medical Biochemistry, Hiroshima University, Graduate School of Biomedical Sciences, Hiroshima, Japan

Synovium-derived mesenchymal stem cells (MSCs) are considered to be a superior source for cartilage regeneration based on their capacity for self-renewal and chondrogenic potential. Although various scaffolds for MSC-based therapy have been investigated for tissue engineering in cartilage repair, these materials usually contain synthetic polymers or animal-derived materials with potential concern about long-term safety. Therefore, we have developed a technology to generate a scaffold-free tissue-engineered construct (TEC) derived from human synovial MSCs.
TEC contains undifferentiated MSCs at high density (initial density: 4×10^5 cells/cm^2) in a three-dimensional matrix which has been synthesized by the MSCs themselves. We have already confirmed that the TEC effectively promote cartilage repair and regeneration in a large animal model. Conventionally, culture media containing fetal bovine serum (FBS) or autologous serum have been used for isolation and expansion of MSCs, and subsequent manufacture of TEC with these MSCs. Such inclusion of serum may have potential risks of prion, viral infection and cross-species immune reactions. In addition, composition of serum is diverse batch-dependently, and this diversity could affect

proliferation and multi-potency of MSCs, and the quality of the TEC. Therefore, there have been the needs for the development of a serum-free culture system.
In this study, we developed a culture system for isolation and expansion of human synovium-derived MSCs, and generation of TEC by using chemically defined serum-free media STK® series: STK1® and STK2® (DS Pharma Biomedical Co., Ltd., Japan). While STK1® is useful for primary cultures from synovial tissue; STK2® is for cell expansion after the 1st passage and subsequent TEC construction.
First, we observed that these serum-free culture conditions allowed rapid attachment and expansion of synovium-derived primary cells. The growth rate and colony-forming efficiency were higher than those under conventional conditions with 10% FBS. Furthermore, MSCs cultured with STK®-series showed higher chondrogenic and osteogenic potential than those cultured with 10% FBS-containing medium. Also, morphological and surface marker expression analysis indicated that MSCs expanded in STK® series media retained MSCs characterization (positive for CD13, CD44, CD73, CD90, and CD166; negative for CD34 and CD45).
Next, with these synovium-derived MSCs and STK2®, we tried to develop the TEC. The TEC could be effectively developed likewise those constructed with 10% FBS-containing medium. The period for manufacture of TEC was reduced by half, up to 20 days. Moreover, total number of constituent cells, cell viability, surface antigen markers for MSCs, the thickness, and chondrogenic potential (glycosaminoglycan quantity, toluidine blue staining, and chondrogenesis marker genes expression) of TEC were all increased or promoted by the use of STK2®.
In conclusion, with STK1® and STK2®, we have established a novel, serum-free culture system for isolation, expansion of human synovium-derived MSCs, and generation of TEC. This method is efficient, time saving, reliable, and safe, and gives a high yield of cells and a high quality of TEC compared to the conventional methods. This novel system will be beneficial for the further step, clinical study for cartilage repair and regeneration.

T-3035

ESTABLISHMENT OF A GFP- POSITIVE IMMORTALIZED MESENCHYMAL STEM CELL LINE EXPRESSING TELOMERASE AFTER LENTIVIRAL GENE TRANSFECTION

Tahereh Karimi[1], Mohamadreza Baghaban Eslaminejad[2], Elham Zomorodian[2], Forough Azam Sayahpour[2]
[1]Department of Biochemistry, School of Veterinary Medicine, Shiraz University, Shiraz, Iran, Islamic Republic of, [2]Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, Academic Center for Education, Culture and Research (ACECR), Tehran, Iran, Islamic Republic of

Introduction:
Combination of special properties of
mesenchymal stem cells (MSCs) including their self-renewal and differentiation potential
into the multiple cell lineages and their readily accessibility from several
adult tissues, made them the major concentration of basic scientists and
clinicians. Since large number of cells must be available for clinical and
basic stem cell studies, MSCs must be expanded after their isolation, but the
finite life span of MSCs due to replicative senescence is a major problem
remained to be solved. Telomere shortening is a molecular mechanism underlying
cellular senescence. Maintaining
stable telomere length is associated with the activation of telomerase, a
ribonucleoprotein complex, containing a catalytic subunit with reverse
transcriptase activity and a RNA component which acts as a telomeric template.
Since there is a close correlation
between telomerase activity and proliferative capacity of eukaryotic cells,
establishment of an immortal cell line by over expression of telomerase may
overcome replicative senescence. Therefore here we attempted to establish an

immortalized mesenchymal stem cell line from rat bone marrow by transfection with human telomerase reverse transcriptase (hTERT) gene.
Methods: MSCs were isolated from bone marrow according to their plastic adherence characteristics. The cells were then trasfected with a Lentiviral vector containing hTERT and GFP genes. Telomerase-expressing, GFP positive MSCs were sorted by anti GFP antibody using a flow cytometer. Expression of hTERT gene and telomerase activity has been confirmed by RT- PCR and Telomerase Repeat Amplification Protocol (TRAP) respectively in transfected cells. The cells were investigated in terms of their karyotype. We also studied biological characteristics of transfected cells including the expression of MSC surface markers, their proliferation capacity and differentiation potential toward mesodermal lineage.
Results: According to our findings transfected cells sustained their normal morphology and karyotype after transfection. The cells expressed MSC markers including CD44, CD90, CD73, CD 105 and did not expressed non-mesenchymal markers including CD45, CD34, and CD11b. Transfected cells showed strong proliferation capacity. Based on our data, GFP-positive telomerase expressing cells maintained their self-renewal and differentiation potential into osteocytic, chondrocytic and adipose cell lineages after 40 passages.
Conclusion: In conclusion, over expression of telomerase in rat BM-MSCs overcome the problem of limited expansion capabilities of MSCs due to cellular senescence. GFP- telomerase positive immortalized rBM-MSC line which sustained their immunophenotyping, normal karyotyping, self-renewal and differentiation potential into mesodermal lineage after 40 passages could be a promising tool for preclinical studies.

T-3036

ISSUES DISTANCE STEM CELL RESEARCH ACHIEVEMENTS FROM BEING APPLIED IN TRANSLATIONAL MEDICINE: A QUALITATIVE STUDY FROM IRAN

Hamid Reza Bidkhori[1], Ahmad Reza Bahrami[5], Rahele Miri[2], Hadi Bakhtiari[1], Mohammad Reza Hedayati-Moghaddam[3], Mahdi Mirahmadi[1], Majid Danaie[4], Mahtab Dastpak[6], Maryam M. Matin[6]
[A]Stem Cells and Regenerative Medicine, [B]AIDS, HTLV and Viral Hepatitis Research Department, [C]AIDS, HTLV and Viral Hepatitis, [D]Social sicences, [1]ACECR-Mashhad Branch, Mashhad, Iran, Islamic Republic of, [A]Cell and Molecular Biology Research Group, Institute of Biotechnology, [B]Department of Biology, Faculty of Science, [2]Ferdowsi University of Mashhad, Mashhad, Iran, Islamic Republic of

Introduction: According to the National Institute of Health (NIH) it is estimated that 80 to 90 percent of potential therapeutics in preclinical testing runs into obstacles that prevent them from being translated to the clinical phase. One of the main reasons is difficulties in developing and sustaining collaborations between basic scientists and clinicians. Translational research based on stem cell therapy, by its nature, crosses boundaries between basic science and clinical application. Crossing these boundaries contributes directly to overcoming challenges to engage basic scientists working on stem cell science in clinical research and practice. The aim of this qualitative study was to identify obstacles in application of basic stem cell science discoveries in opinion of Iranian scientists and clinicians.
Materials and Methods: Sixteen experts in stem cell science and/or specialists in different clinical fields, working as distinguished academic members at universities and research centers of Mashhad, Northeastern Iran, were selected by purposive sampling method. Each deep interview was recorded with the permission of the interviewee and converted to text. Thematic analysis with open coding was done using MAXQDA ver. 10 software and axial codes were classified based on factors related to Inefficiencies of basic stem cell researches.

Results and Conclusion: A content analysis identified eight themes and 21 subthemes that described the participants' opinions on the major drawbacks of translational medicine in Iran. The major themes were: insufficiency of clinical trials regarding stem cells; unidentified ethical and legal concerns in different steps of translation; poor level of understanding each other between basic scientists and clinicians or lack of common language between them; differences between their professional environments, and heterogeneous areas of expertise; and different end-points in their work. This creates considerable problems of communication.
We also discuss here the possible ways to overcome the obstacles by providing suggestions that may enable this collaboration and highlight strategies to improve the effective communication. Some of them are as below: governmental research investments and academic and industrial grants should involve both groups in benefits and responsibilities of translational research and must be increased in amount; it is recommended to admit medical students who have B.Sc. degree, as this is considered as the golden period to lay the basis for knowledge based researches and that the efforts to entice admitted medical students and residents to do research are too little too late and should be up scaled; more emphasize and priorities should be allocated to MD/PhD programs to fill the gap of understanding; in certain fields the regular MD/PhD trend could be reversed by introducing of PhD/MD or encouraging physicians with different specialties to enroll PhD degree or fellowship programs for cell therapy. Some other suggestions in the article include ways for elimination of the barriers exists in translational research. It is regarded as a starting point to encourage debate on this issue.

T-3037

THERAUTIC EFFECTS OF TIME WINDOW FOR HUMAN UMBILICAL CORD BLOOD-DERIVED MESENCHYMAL STEM CELLS WITH METHYLPREDISOLONE TREATMENT IN THE CONTUSED RAT SPINAL CORD

Sang In Park
Institute of Catholic Integrative Medicine,, Incheon St.Mary's Hospital, The Catholic University of Korea College of Medicine, Incheon, Korea, Republic of

Therapeutic effects of time window for human umbilical cord blood-derived mesenchymal stem cells with methylprednisolone treatment in the contused rat spinal cord

Sang In Park[a], Yoonjin Bae[a], Hyun-joo Ham[a], Yu Jin Jung[a], Moon-Seo Park[a], Yong-An Chung[a], Lee-So Maeng[a], Woochul Chang[a]

[a]*Insitute of catholic Integrative Medicine (ICIM), Incheon St. Mary's Hospital, The Catholic University of Korea.*

Abstract
Methylprednisolone (MP), a glucocorticoid steroid, has an anti-inflammatory action and seems to inhibit the formation of oxygen free radicals produced during lipid peroxidation in a spinal cord injury (SCI). Currently MP is the standard therapy after acute SCI on reported neurological improvements. The combination therapeutic effect of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) for transplantation time (1d, 7d, and 30d) after MP treatment on the axonal regeneration and on the behavioral improvement in SCI were studied in the rat. The spinal cord was injured by contusion using a weight-drop at the level of T9 and MP (30 mg/kg, i.m., 10 min and 4 h) was acute administered after injury. hUCB-MSCs were labeled GFP and our study was performed the efficacy for transplantation time (1d, 7d, and 30d) of hUCB-MSCs into the boundary zone of injured site. Efficacy was determined by histology, anterograde and retrograde tracing, and behavioral test. We found that hUCB-MSCs with MP treatment exerted a significant beneficial effect by neuroprotection and reducing cavity volume. Also the transplantation of hUCB-MSCs with MP treatment was significantly improved functional recovery. Combined transplantation at 7d after SCI provided significantly greater efficiency than combined transplantation at 1d and 30d. These results suggest that transplantation time window of the hUCB-MSCs with MP treatment give rise to an earlier neuron protection strategy and effect of cell grafting in SCI. Thus our study may be considered as a therapeutic modality for SCI.

Mesenchymal Cell Lineage Analysis

T-3043

REJUVENATION OF HIGH PASSAGE MESENCHYMAL STEM CELLS BY CULTURE IN HUMAN PLATELET LYSATE

Sarah Griffiths[1], Priya R. Baraniak[2], Ian B. Copland[3], Robert M. Nerem[1], Todd C. McDevitt[2]

[A]Parker H. Petit Institute of Bioengineering and Bioscience, [B]The Wallace H. Coulter Department of Biomedical Engineering, [1]Georgia Institute of Technology, Atlanta, GA, United States, [2]Department of Hematology and Medical Oncology, Emory University, Atlanta, GA, United States

Mesenchymal stem cells (MSCs) are a promising source for various cellular therapies and current *ex vivo* expansion techniques rely upon xenogenic products, such as fetal bovine serum (FBS), to supplement culture media. Recently, human platelet lysate (hPL) has been shown to maintain the differentiation potential and immunophenotype of MSCs when expanded to clinically relevant cell numbers. hPL also alleviates the legitimate concerns over the inadvertent transmission of potentially harmful animal pathogens. The objective of this study was to determine the effects of hPL on human bone marrow MSCs following different periods of FBS exposure. MSCs were cultured in medium containing either FBS (16%) or hPL (5%) for up to 16 passages and within this study we determined the cell size, doubling time and senescence, via a fluorometric assay for endogenous beta-galactosidase. MSCs cultured with FBS for different numbers of passages were switched to hPL conditions and the ability of hPL to rejuvenate the proliferative capacity of MSCs was evaluated. hPL resulted in significantly ($p<0.01$) more rapid cell proliferation at earlier passages ($p\leq5$) than FBS, by day 4 of culture hPL yielded an MSC doubling time of 1.28 days compared with 1.52 days in FBS. MSCs cultured in hPL demonstrated a significantly smaller cell size ($p<0.001$) than in FBS cultures and were more homogeneous demonstrated by significantly less deviation in cell size ($p<0.001$). Early passage (p6-8), late passage (p10-11) and senescent (>p13) MSCs cultured first in FBS and then switched to hPL proliferated more and demonstrated less β-gal production and smaller cell sizes than MSCs continuously propagated in FBS. These data demonstrate that culture of bone marrow-derived MSCs in hPL enhances cell proliferation and attenuates cell senescence compared to culture with FBS. Moreover, recovery of senescent or aged MSC populations may be possible by exposure to hPL. The results of this study may enable more effective clinical-scale expansion of MSCs than current *ex vivo* culture techniques due to attainment of clinical cell numbers after shorter *in vitro* culture durations.

T-3044

HUMAN UMBILICAL CORD STROMA-DERIVED STEM CELLS FROM FOUR DIFFERENT TISSUE SUBCOMPARTMENTS HAVING DISTINCT STEMNESS PROPERTIES RETAIN THEIR CHARACTERISTICS WHEN ISOLATED USING EXPLANT CULTURES

Deniz BALCI[1], Alp CAN[2]

[1]Ankara University Biotechnology Institute, ANKARA, Turkey, [2]Department of Histology & Embryology, Ankara University School of Medicine, ANKARA, Turkey

Human umbilical cord stroma-derived mesenchymal stem cells (hUCS-MSCs) are considered as a promising type of MSCs to be successfully used in cellular therapies. When isolated by an enzymatic procedure and cultured on plastic surfaces, hUCS-MSCs in vitro show a highly heterogeneous phenotype and differentiation potential suggesting that these populations may have different stemness grades. We have previously shown that in vivo protein profiles are dramatically changed when cells were extracted from tissues of origin using enzymatic digestion procedures. In present study, we demonstrate varying characteristics of hUCS-MSCs originated from different UC compartments that have been differentially isolated using nonenzymatic procedure. Perivascular compartment (PVC), intervascular compartment (IVC), subamnion (SA), and arteries (UCA) were carefully excised, and then explanted onto plastic culture flasks. Seeded cells in the following weeks/months were analyzed through P0 to P13. Surface markers were determined by the end of P0. Cell morphology, proliferation dynamics, major cytoskeletal protein distribution and differentiation potentials were differentially evaluated during P1-P13. Among four explant cultures SA has the highest and the fastest seeding rate that reached to confluency at 9 days in P0. IVC, UCA and PVC showed slower confluency rates; 15, 17 and 22 days, respectively. The most heterogeneous group of cells was noted in IVC containing two major cell phenotypes (type 1 and type 2). PVC cells were smaller and more fusiform; whereas SA

cells displayed more epithelial phenotype. Distinct from the others UCA cells were slender and homogeneous. Surface marker analyses demonstrated that CD90 was the only common marker (>90%) for all groups. Second most common markers were CD29 and CD105 that were higher (>90%) in IVC and SA compared to PVC (73-76%) and UCA (83-90%). Depending on the CD markers analyzed, PVC cells exist as a distinct population among the other groups. The distribution of cytoskeletal proteins in all cells from four compartments was then documented as compared to their tissue of origin. Desmin was the only protein that diminished (excluding UCA cells) when IVC, PVC and SA cells were cultured during P2-P5. ACTA2, vimentin, cytokeratin (CK) and CD146 (NCAM) profiles showed varying degrees of positivity throughout P1-P13. Specifically, CK was dominantly found in young cells, while ACTA2 were prominent in giant cells. In all groups, cells entered to replicative senescence after P8; overall population-doubling time (PDT) was shortest in PVC cells (40 h at P1-P8 and 51 h at P9-P13) and longest in IVC (49 h at P1-P8 and 68 h at P9-P13). UCA cells remarkably entered to senescence earlier than the other cells. PVC cells were differentiated into adipocytes more effectively than the other groups, while SA and IVC had more potential to osteogenic differentiation. Interestingly, in many aspects, arterial wall smooth muscle cells responded similar to stromal MSCs. Conclusively, tissue compartments within the human UC possess varying degrees of stem cell properties as indicated by the present study that PVC cells have higher proliferation rate and are more potent in differentiation to adipocytes, whereas IVC and SA cells have slower rate of proliferation but have a higher response to osteogenic stimuli. As cells from four tissue compartments displayed distinct cellular features they should not be considered as a cohort in tissue engineering and cell-based therapies.

T-3045

A NOVEL POPULATION OF IMMUNOREGULATORY DERMAL PROGENITOR CELLS

Tobias Schatton[1], Jun Yang[3], Sonja Kleffel[1], Natasha Frank[2], Ana Maria Waaga-Gasser[4], Martin Gasser[4], Thomas Kupper[1], Mohamed Sayegh[3], **Markus Frank**[3]
[A]Dermatology, [B]Medicine, [1]Brigham and Women's Hospital, Boston, MA, United States, [2]Medicine, Boston Children's Hospital, Boston, MA, United States, [3]Surgery, University of Würzburg, Würzburg, Germany

Stem cell-based immunomodulatory strategies represent a new therapeutic frontier in allotransplantation. However, identification of specific molecular markers for the isolation and expansion of effective immunomodulatory stem cell subsets, and dissection of the molecular mechanisms involved, require further investigation. Here, we have isolated and characterized a novel dermal progenitor cell (DPC) subset from murine skin based on expression of the ATP-binding cassette transporter, Abcb5, the murine homologue of human ABCB5, which also marks progenitor cells in human skin. In ex vivo immune activation assays, both syngeneic and allogeneic Abcb5(+) DPCs suppressed alloantigen- and mitogen-dependent T cell proliferation compared to controls. Fluorescent cell tracing and flow cytometric analysis revealed that allogeneic DPCs evade immune rejection, home to recipient immune tissues, and induce CD4(+)CD25(+)Foxp3(+) regulatory T cells (Tregs) in vivo. In fully MHC-mismatched murine heterotopic cardiac allotransplantation models, treatment with donor- or third party-strain purified DPCs resulted in significantly ($P<0.001$, respectively) prolonged cardiac allograft survival compared to untreated controls (median survival time: 19.0 vs. 7.5 and 24.5 vs. 9.0 days, respectively). Additionally, Treg frequencies were significantly increased in cardiac allografts dissected from recipients of either donor- or third party-strain DPCs. Systematic examination of the immunoregulatory pathway repertoire of Abcb5(+) DPCs showed marked expression of the immunologic checkpoint, programmed death 1 (PD-1). Mechanistically, DPC-specific shRNA-mediated knockdown of PD-1 reversed the inhibitory effect of DPCs on alloantigen- and mitogen-dependent T cell proliferation and suppressed DPC-mediated Treg induction. Our results establish immunomodulatory functions of Abcb5(+) DPCs and point to promising roles of this novel progenitor cell subset in cell-based immunotherapeutic strategies.

T-3046

IMMUNE PROPERTIES OF HUMAN CD200$^+$ SUB-POPULATION FROM PLACENTA-DERIVED MESENCHYMAL STEM CELLS

LIU TING, WANG Li-bin, ZHU Yong-zhao, MA Xiao-na, LI Yu-kui, WEI Jun
Ningxia Human Stem Cell Institute, the General Hospital of Ningxia Medical University, yinchuan, China

Background: MSCs modulate the immune function of the major immune cell populations involved in alloantigen recognition and elimination, including antigen presenting cells, T cells, B cells and natural killer cells. Because of this aspect, MSCs can be clinically applied for graft-versus-host and autoimmune diseases. The placenta has been suggested as an abundant, ethically acceptable, less immunogenic and easily accessible source of MSC. Objective: Because of PMSC prepared from different laboratories exhibit significant heterogeneity, we aimed to identify and isolate a sub-population of PMSC with high positive surface antigen about CD200. We hypothesized that increased CD200 positive cell percent contributes to regulate the immune response. Methods and Results:The PMSCs were isolated and cultured in the normal procedure. Flow cytometry sorting was used to isolate the CD200 positive and CD200 negative sub-population of PMSCs. The phenotype analysis showed that the cell surface marker CD73, CD90, CD105 was positive, while CD80, CD86, CD34 and HLA-DR was negative in both CD200 positive and CD200 negative sub-population cells. Differentiation of CD200 positive and CD200 negative sub-population towards adipogenic and osteogenic lineages was performed in vitro. The results indicate that CD200 positive and CD200 negative sub-population could be differentiated into not only mesenchymal, but also ectodermal lineage. The mixed lymphyocyte proliferation assays were performed using standard methods. Mononuclear cells were acquired from peripheral blood by density gradient centrifugation. The CD200 positive and CD200 negative sub-population were co-cultured with TLR ligand-activated blood mononuclear cells, and the immunomodulatory functions of the CD200 positive and CD200 negative sub-population were evaluated by suppressed secretion of IL-6, IL-8, IFN-A, and TNF-A by ELISA. The peripheral blood mononuclear cells of acute respiratory infected patients were using to detected the immunomodulation of CD200 positive sub-population from PMSCs. Results: The PMSCs CD200 positive sub-population could not induce the proliferation of lymphocytes. The proliferation of lymphocytes could be inhibited to different extents by adding different ratios of CD200 positive sub-population.Meanwhile, The PMSCs CD200 positive sub-population could reduce immunoreactions factors in the mixed lymphocyte reaction experiment. Conclusions:Compared with PMSCs and CD200 negative sub-population of PMSC, The CD200 positive sub-population from PMSCs maintained MSC phenotype and differentiation potential, while expressed increased immunomodulatory functions.

T-3047

LINEAGE SPECIFICITY DETERMINES THE MYOGENIC AND IMMUNOREGULATORY BUT NOT ANGIOGENIC CAPACITIES OF SUBPOPULATIONS OF BLOOD-VESSEL-DERIVED STEM CELLS

Chien-Wen Chen[1], Arman Saparov[2], Minakshi Poddar[1], Seth D. Thompson[1], Bruno Péault[1], Johnny Huard[1]
[1]Univ of Pittsburgh, Pittsburgh, PA, United States, [2]School of Science and Technology, Nazarbayev University, Astana, Kazakhstan

There is increasing evidence indicating the perivascular origin of MSCs and the association between tissue-specific stem/progenitor cells and vascular niches. We and other researchers have recently identified and characterized distinct subpopulations of multipotent precursors from definitive structural layers of blood vessels in human skeletal muscle. These subpopulations of blood-vessel-derived stem cells (BVSCs) not only express unique cell surface marker profiles but also display classic mesenchymal stem/stromal cell (MSC) markers. In particular, myogenic endothelial cells (MECs, CD56+/CD34+/CD144+/CD45–) and pericytes (PCs, CD146+/CD34−/CD45−/CD56−), derived from microvascular intima and stroma respectively, have been shown to exhibit robust myogenic and tissue repair capacities in separate studies, in addition to their typical mesodermal developmental potentials. Despite the seemingly common differentiation capabilities, we hypothesize that the lineage origin and/or commitment of these BVSC subpopulations specifies their myogenic and regenerative potentials. MECs and PCs were simultaneously purified to homogeny from a single human skeletal muscle biopsy by FACS and cultured for 5-6 passages. To examine the influence of lineage specificity on myogenesis, cells were expanded until confluence before switching to myogenic culture conditions (2% serum) *in vitro*. Human umbilical vein endothelial cells (HUVECs) were used as a typical EC control, replacing the phenotypically unstable muscle-derived ECs. When cultured in myogenic conditions for

1 week, MECs exhibited the greatest extent of multi-nuclei myotube formation (P<0.01). PCs formed significantly less and smaller myotubes while nearly all HUVECs were extinct in the myogenic culture, confirmed by DAPI staining. Immunocytochemical analyses of myogenic markers revealed that MECs not only mostly displayed the early myogenic marker, desmin, but also extensively express mature myogenic markers, fast and slow skeletal muscle myosin heavy chain (FS-MHC and SS-MHC). A notably smaller proportion of PCs express desmin, FS-MHC, and SS-MHC, when compared to MECs (all P<0.05), suggesting a smaller extent of myogenesis and lesser maturation. To reveal the myogenic capacity *in vivo*, cells were injected into cardiotoxin-injured hind-limb muscles of immunodeficient mice for a duration of 3 weeks. Immunohistochemical studies showed that MEC-injected muscles exhibit the supreme regeneration of human-specific spectrin myofibers, compared to all other groups (P<0.05). Additionally, transplantation of MECs and PCs both promoted host CD31+ microvascular density to a similar level at the injury site, suggesting that their angiogenic capacities were not influenced by the differential myogenesis. To investigate the immunoregulatory responses of MECs and PCs in the cardiotoxin-injured muscle, we simulated oxidative and inflammatory stresses by adding hydrogen peroxide (250μM) and LPS (1μg/ml) respectively into the cell culture and incubated for 24 hours. ELISA revealed that under oxidative and inflammatory stress, MECs and PCs produced similar levels of MCP-1, TGF-β, and IL-1β but no IL-10 and IL-12 while MECs secreted a notably higher level of IL-6 than PCs, suggesting unique immunoregulatory profiles of BVSC subpopulations. Overall, these results suggest that MECs and PCs exhibit distinct regenerative responses, presumably determined by their lineage origin/commitment.

T-3048

REPROGRAMMING OF OXIDATIVE STRESS RESPONSES IN HUMAN INDUCED PLURIPOTENT STEM CELLS DERIVED FROM MENSTRUAL BLOOD MESENCHYMAL CELLS

Regina Goldenberg, Karina Dutra Asensi, Rodrigo Soares Fortunato, Danubia Silva Santos, Deivid Carvalho Rodrigues, Antonio Carlos Campos de Carvalho, Denise Pires Carvalho, Adriana Bastos Carvalho
Federal Univ of Rio de Janeiro, Rio de Janeiro, Brazil

Aim: Properties of induced pluripotent stem cells (iPSC) have been extensively studied since their first derivation in 2006. However, the effect of reprogramming on oxidative stress (OS) responses still needs to be further examined. The objective of this work was to compare the metabolic properties of iPSC generated from menstrual blood-derived mesenchymal stem cells (mb-iPSC) to embryonic stem cells (ESC) and to adult menstrual blood-derived mesenchymal stem cells (mbMSC), investigating the effects of reprogramming on the capacity to modulate OS.
Methods and Results: mbMSC were extremely resistant to reactive oxygen species (ROS). However, mb-iPSC presented a 10-fold decrease in their IC_{50} upon exposure to H_2O_2, which became very similar to ESC IC_{50}. Extracellular production of ROS was also similar in mb-iPSC and ESC and almost 3-fold lower than in mbMSC. Since NADPH oxidases are important contributors to extracellular ROS production, we investigated their expression by quantitative RT-PCR. NOX2 was expressed in all cell types, with no differences between them, while NOX5 was expressed only in mbMSC. Intracellular ROS production was also studied using DCF assay. No differences were found in the amount of ROS produced in the cytoplasm of these cells in baseline conditions. Furthermore, addition of H_2O_2 led to a significantly higher increase in the intracellular amounts of ROS in mb-iPSC and ESC when compared to mbMSC. As the ability to metabolize ROS is related to antioxidant enzymes, we analyzed enzyme expressions and activities in these cell types. mb-iPSC and ESC presented around 600-fold lower expression of catalase when compared to mbMSC. Superoxide dismutase (SOD) 2 and 3 mRNA was also lower in mb-iPSC and ESC compared to mbMSC, in a range of 14- to 31-fold reduction. SOD 1 reduction was more discrete and very similar in mb-iPSC and ESC, while glutathione peroxidase (GPx) 1 was 16-fold lower in mb-iPSC and 6-fold lower in ESC. In accordance to quantitative RT-PCR results, catalase activity was reduced in mb-iPSC and ESC. SOD activity was also reduced in mb-iPSC and ESC, while GPx activity was similar in these cells. Finally, cell adhesion under oxidative stress conditions was impaired in mb-iPSC when compared to mbMSC, albeit similar to ESC. At 200 μM of H_2O_2, adhesion of ESC and mb-iPSC was significantly impaired, while mbMSC maintained adhesion in similar levels to control. Above 400 μM, adhesion was compromised in all cell types, even though the percentages of reduction were more pronounced in pluripotent stem cells.

Conclusion: Thus, reprogramming leads to profound modifications in cell metabolism with loss of the capacity to modulate OS. Our work demonstrates that the reduction in oxidative metabolism is not accompanied by increases in antioxidant defenses in induced pluripotent stem cells, indicating an important mechanism by which these cells could maintain low levels of damage coupled with a high sensitivity to changes in oxidative stress.
Sources of research support: CNPq, Capes, FAPERJ, Ministério da Saúde
Key words: Menstrual blood stem cells, mesenchymal stem cells, oxidative stress.

T-3051

MENSTRUAL STEM CELLS OUT-PERFORM BONE MARROW MESENCHYMAL STEM CELLS IN PROLIFERATION, MIGRATION RATES AND SUPPORT OF HEMATOPOIETIC STEM CELL EXPANSION.

Francisca Alcayaga-Miranda, jimena Cuenca, Patricia Luz-Crawford, Maroun Khoury
Faculty of medicine, Laboratory of miRNA and stem cells, Universidad De Los Andes, Santiago, Chile

INTRODUCTION. A highly proliferative stem cell was recently identified in menstrual fluid. The menstrual stem cells (MenSC) exhibited mesenchymal stem cell (MSC)-like properties and some apparent culture and expansion advantages. The aim of this study was to characterize this new population of MenSC, compare it to the broadly studied bone marrow derived stem cells (BM-MSC) and finally test their ability to support hematopoietic stem cells (HSCs) expansion in vitro. METHODS. MenSC were isolated and characterized under standard conditions according to surface markers expression and tri-lineage differentiation potential. Cell proliferation and viability were determined by measuring cellular mitochondrial dehydrogenase activity. Cell migration was evaluated in an in vitro scratch assay and cord blood HSCs were directely co-cultured on MenSC and BM-MSC or in a transwell in a growth factor-defined medium, and then tested for their distinct colony formation potential. RESULTS. Although, no difference in cell surface markers was observed, the proliferation rate, the CFU-F numbers and migration capacity of the MenSC were significantly higher in comparison to BM-MSC. CFU-F capacity from MenSC was 4X higher than BM-MSC and the complete closure of the scratch in the migration assay was achieved at 24±2-3 hours in MenSC versus 48±8h in BM-MSC. More interestingly, we have demonstrated for the first time, the capacity of MenSC to support ex-vivo expansion of HSCs. At 7 days post-culture, HSCs showed a 3X higher expansion rate of the CD34+CD133+ population, in the presence of MenSC and in comparison to other culture conditions. Furthermore, we determined that this proliferation is cell-to-cell contact mediated. Additionally, expanded HSC on MenSC-feeder showed 3 folds higher number of CFU-GEMM colonies. CONCLUSION: MenSC are a unique stem cell population that can be easily and periodically obtained, isolated and cultured. In this study we have demonstrated that these cells are superior to BM-MSCs for their proliferation rate, CFU-F, migration capacity and support of cord blood HSCs. These results point at the potential of the MenSC as a new source of feeder cells for HSC expansion and for clinical applications.

T-3052

MCP-1 INDUCED MESENCHYMAL STEM CELL MIGRATION IS DEPENDENT ON GPCR βγ SUBUNIT, PI3K, RAC AND RHO SIGNALING

Caroline Ryan[1], Aine Prendergast[2], James Brown[1], Timothy O' Brien[1], Frank Barry[1]
[1]REMEDI, NUI Galway, Galway, Ireland, [2]DKFZ, Heidelberg, Germany

Mesenchymal stem cells (MSCs) have generated much interest in recent years because of their therapeutic potential in a broad spectrum of diseases. The capacity of MSCs to migrate to a site of injury and engraft is central to their ability to contribute to a repair response. Chemotaxis, the directed movement of cells along extracellular chemokine gradients, is a critical cellular response for repair of damaged host tissue.
However, the molecular mechanisms and chemotactic signaling events that guide MSCs to their appropriate microenvironments have not been fully elucidated. Understanding these mechanisms will advance the therapeutic utility of MSCs through improved methods for systemic delivery, more efficient homing to targeted tissues and enhanced engraftment and retention.

We found that MSCs rapidly migrated in response to a gradient of MCP-1. While the role of MCP-1 as an injury-specific chemoattractant for MSCs has been described, the precise mechanisms governing the migration of MSCs in response to MCP-1 have not been well-resolved. Firstly, we aimed to characterise the cellular mechanics mediating MSC migration. Using high-resolution confocal microscopy and a live cell system incorporating a pre-formed gradient of MCP-1, we found that directional migration was supported by a polarised phenotype and distinct changes in actin and α-tubulin dynamics. By using a specific inhibitor for the Rho kinase, ROCK we found that ROCK is essential for actin mediated tail retraction and lamellipodia formation in MSCs. We next aimed to elucidate the complex array of intracellular signals that may mediate migration of MSCs in response to MCP-1. By using defined inhibitors to perturb receptor activation and specific points of the downstream signalling cascades we have deciphered the critical mediators of migration in MSCs. We define a role for PI3K, ERK1/2 and the Rac effector, PAK in MCP-1-induced MSC migration. A detailed analysis of the MCP-1 receptor, CCR2 revealed that redistribution and clustering of CCR2 on the surface of MSCs precedes intracellular co-localisation and clustering of the CCR2 adapter molecule FROUNT. CCR2 and FROUNT clustering is essential for downstream signaling and resultant migration and is dependent on the βγ subunits of GPCRs. These findings suggest that CCR2 transmits chemotactic signals through Gβγ and PI3Kγ in MSCs. Together these results provide novel insights into the important mechanisms orchestrating MSC migration. By following a systematic approach we have been able to map a path of MSC migration to a therapeutically relevant chemokine. As a result we have identified potential targets in MSCs that could be exploited to improve homing and retention of MSCs in injury or disease settings.

T-3053

COMPARING THE VIABILITY AND GROWTH IN CULTURE OF STEM CELLS OBTAINED FROM ADIPOSE TISSUE (ADAS) PRE AND POST CRYOPRESERVATION

Luz Mabel Avila Portillo, Derly Franco, Jenniffer Priscila Avila, Angela Riveros, Mauricio Sabogal
Regenerative Medicine Stem Lab, Bogota, Colombia

Introduction
Studies *in vitro* and clinical assays are demonstrating
that stem cells form ADAS are remarkable alternative for regenerative medicine.
There for, the ADAS cryopreservation storage method has to be optimized to guaranty
its future application.
Objective
Compare viability,
growth in culture and ADAS phenotype pre and post cryopreservation with two
cryoprotector solutions.
Methods
Previous consent information signatures were obtained.
12 samples of adipose tissue of healthy individuals who had liposuction, used enzyme for the digestion of tissue fat prior to cell isolation The vascular stromal fraction obtained was equivalently divided in two fractions: The fraction 1 was cryopreserved and stored at -196 C and fraction 2 was cultivated in DMEM/LG, LPRP 10% until passage 1 (confluence 90% in T25 (ttp®)). Cells were trypsinized, count and viability performed by Trypan blue and equivalently divided in two fraction (3 and 4), the fraction 3 was characterized by Flow cytometry with CD34, CD45, CD90, CD105, CD73 and the cells were differentiated to osteogenic linage (Stem Cell Technologies). Fraction 4 was frozen in a similar way as
fraction 1. Fraction 1 and 4 were cryopreserved by means of automatized
freezing chamber (Thermo CryoMed Freezer) utilizing two types of freezing
solutions: DMSO/DEXTRÁN/Albumin (A) and DMSO/HESSICO/Albumin (B) and stored for 3 months at -196 °C. Both fractions were
thawed with PBS/Albumin/HESSICO and subsequently the samples were submitted to culture, characterized by Flow cytometry and differentiated to osteogenic linage similar to fraction 2.
Results

The average age of individuals was 35 years, which 75% were woman and
25% man. The average of CM obtained per gram of fat was 6,93x10^5CMN.
The cryopreserved Fraction 1 with solution A showed 5,23x10^5 CMN and 34% of viability and with solution B showed 5,22x10^5 CMN and 25%
of viability. Both subfractions reach a confluence of 90% in 25
days of culture. The cryopreserved fraction 4 with solution A showed
62% of viability and 6 days of culture to reach a confluence of 90%. Meanwhile, cryopreserved fraction 4 with solution B showed
35% of viability and reach a confluence of 90% in 9 days of culture. Both subfractions showed 6x10^5 ADAS. The expanded samples did not show phenotypic differences by flow citometry and both showed osteogenic linage differentiation. The fraction 2 cultured previously to freezing with an initial graft of 5,0x10^3CMN/cm^2 reached a confluence percentage of 90% in day 10 of culture and its expansion capacity was not affected, obtaining
14x10^6ADAS in the first passage.

Discussion

This study suggests that the cryopreservation solution DMSO/DEXTRÁN/Albumin could be a better option to store ADAS and its culture previous to freezing increases its viability percentage. The 3 fractions of ADAS maintain phenotype and osteogenic linage differentiation capacity.

T-3054

AGING BONE MARROW MESENCHYMAL STROMAL CELLS HAVE ALTERED MEMBRANE GLYCEROPHOSPHOLIPID COMPOSITION AND FUNCTIONALITY

Lotta Kilpinen[1], Feven Tigistu-Sahle[2], Sofia Oja[1], Dario Greco[3], Amarjit Parmar[4], Päivi Saavalainen[5], Janne Nikkilä[1], Matti Korhonen[1], Petri Lehenkari[6], Reijo Käkelä[2], **Saara Laitinen**[1]

[1]Advanced Therapies, Finish Red Cross Blood Service, Helsinki, Finland, [2]Biosciences, University of Helsinki, Helsinki, Finland, [3]Finish Institute of Occupational Health, Helsinki, Finland, [4]Medical Genetics, Hartman Institute, Helsinki, Finland, [5]Medical Genetics, Hartman Insititute, Helsinki, Finland, [6]Institute of Clinical Medicine, University of Oulu, Oulu, Finland

Background

Human mesenchymal stem/stromal cells (hMSCs) are increasingly used in advanced cellular therapies. The clinical use of hMSCs demands sequential cell expansions and since it is well established that membrane glycerophospholipids (GPLs) provide precursors for signaling lipids that modulate cellular functions, we studied the effect of the donor's age and
cell doublings on the GPL profile of human bone marrow MSC (hBMSC). Aim

The aim of our study was to compare the GPL profiles of hBMSCs from young and old donors, study the effects of sequential expansion of the cells on these profiles, determine the expression of genes related to lipid metabolism and immunomodulation, and gain a better understanding
of the possible relations of cell lipidome changes, aging and functionality. Results

The hBMSCs, harvested from 5 young adult and 5 old donors, showed clear compositional changes during expansion, seen at the level of lipid classes, lipid species and acyl chains. The ratio of phosphatidylinositol (PI) to phosphatidylserine (PS) increased towards the late passage samples. Furthermore, arachidonic acid (20:4n-6) containing species of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) accumulated while the species containing monounsaturated fatty acids (FAs) decreased during passaging. Additionally, in the total FA pool of the cells 20:4n-6 increased, which happened at the expense of n-3 polyunsaturated FAs (PUFAs), especially docosahexaenoic acid (22:6n-3). The GPL and FA correlated with the decreased immunosuppressive capacity of hBMSCs during expansion. Our observations were further supported by alterations in the gene expression levels of several enzymes involved in lipid metabolism and immunomodulation. Conclusion

Our study is the first to show major changes in phospholipid profiles and total FAs during expansion and senescence of therapeutically important hBMSCs. We demonstrate that the 20:4n-6 contents of cellular lipids increased while the n-3 PUFA contents decreased during long-term cultivation. The gene expression differences were most notable between the early passage cells from the old and the young donors and supported
our lipid findings. Our results suggest that the free PUFAs derived from
membrane lipids are of high importance in hBMSC immunological functionality. Since PUFA-derived signaling lipids are known to be involved in MSC functionality, but are extremely challenging to use as biomarkers, instead, biosynthetic precursors in membranes could be used as indicators of cell functionality. In combination with additional markers, such as mRNA of lipid enzymes and/or other functional factors, membrane lipids can serve as powerful new biomarkers for the functionality of therapeutic cells.

T-3055

GENE EXPRESSION PROFILING OF HUMAN-UMBILICAL CORD DERIVED MESENCHYMAL STEM CELLS (HUC-MSC) DEPENDING ON PASSAGE UNDER MODIFIED MSC CULTURE MEDIUM

Hyun Woo Lee[1], Su-Youne Han[1], Hyeon Kyeong Lee[1], Jin Tac Kim[1], Back Soo Shin[1], Seung Hoon Pak[1], Ji Hyang Kim[1], Jong-Hoon Kim[2], Byung-Rok Do[1]
[1]Biotechnology Reserch institute, Hurim Biocell Inc., Seoul, Korea, Republic of, [2]Laboratory of Stem Cell Biology, Division of Biotechnology, College of Life Scieces and Biotechnology, Korea University, Seoul, Korea, Republic of

hUC-MSCs are regarded as the source of regenerative medicine especially for hepatocirrhosis. However there were various types of cells that isolated from umbilical cord without application of the selective isolation method. Highly purified stem cell isolation, stable expansion and maintenance of intrinsic characteristics of their cells are necessary for clinical application. In this study, we analyzed differential gene expression patterns of mono-nucleated cells isolated from human umbilical cord according to their passage and estimated their cytogenetic character for 5 passages under modified Lab-made MSC culture media. UC-MSCs were characterized with the cell surface marker (CD31, CD34, CD45, CD90) expression using flow cytometry. Multi-potency of hUC-MSC (fat, bone, cartilage, liver) was also confirmed. Expression profiling of hUC-MSCs was analyzed by gene microarray methods (HT-12 array) according to their passages. Exp1) according to their long term culture passage. Exp2) according to their sources; between hUC-MSC and AT-MSC. Exp3) according to w/ or w/o in vitro expansion culture. In exp1, about 300 genes were shown different expression pattern. In exp2), about 1400 genes s howed different expression between hUC-MSCs and AT-MSCs. In exp3), expression patterns of about 3000 genes from freshly isolated hUC-MSCs were significantly different with long term cultured hUC-MSCs. In exp1 and exp2, mRNA expression of MSC related genes (ADAM12, CD31, CD73, CD90, CD105, HLA class I/II-DR) were not changed between groups. Also markers of mesenchymal stem cells (CD73, CD90, and CD105) were stable expressed during long term culture. Especially, the expression of CD90 was over 90 percentages. Also chromosome abnormality was not detected at final passages. Endothelial (CD31), hematopoietic (CD34) and immunogen (HLA class II-DR) related genes were expressed in primary isolated cells, but the expression of CD31, CD34 and HLA class II-DR was decreased according to the culture in exp3. Stem cell related gene (ADAM12, CD90) was increased depending on culture period. These data indicate that isolated mono-nucleated cells from umbilical cord was composed various types of cells but hUC-MSCs were highly purified by in vitro expansion under modified Lab-made MSC culture medium. These results could be used as standard purifying method of hUC-MSCs for clinical application.

T-3056

IN VIVO AND IN VITRO FATE MAPPING STUDIES OF SKELETAL MESENCHYMAL STEM CELLS

Daniel L. Coutu, Timm Schroeder
Stem Cell Dynamics Research Unit, Helmholtz Center Munich, Munich/Neuherberg, Germany

Skeletal or mesenchymal stem cells (MSCs) have been widely studied for their therapeutic potential. However, and despite recent efforts, their ontogeny, fundamental physiology and identity are still highly debated, hindering the development of successful MSC-based therapies. Moreover, few studies have investigated the biology of MSCs from a skeletal and developmental perspective. To address these issues, we use flow cytometry as well as in vitro time lapse microscopy and in vivo fate mapping to elucidate the identity and lineage hierarchy downstream of murine skeletal MSCs. Flow cytometry experiments using published markers of MSC subpopulations demonstrate that these populations overlap only partially. These results were confirmed by spectral scanning confocal microscopy of bone sections. Cell sorting and time lapse microscopy experiments demonstrated that many, if not all of these populations contribute to the heterogeneity of typical MSC preparations in non-clonal cultures, for many passages. To further elucidate the relationship between these subpopulations, we performed in vivo fate mapping experiments using the CreERT2 system. Flow cytometry and confocal microscopy was performed at up to 42 days after tamoxifen administration. To facilitate data analysis, we developed novel algorithms for easier visualization of multidimensional and time resolved flow cytometry data. We are now investigating whether the developmental sequence we observed in postnatal animals recapitulates that of embryonic bone development. In conclusion, the use mouse genetic techniques combined with multicolor flow cytometry and spectral scanning confocal microscopy are useful to decipher the ontogeny and differentiation lineage hierarchy of skeletal MSCs.

Mesenchymal Stem Cell Differentiation

T-3057

AGE-RELATED CHANGES IN THE SYSTEMIC ENVIRONMENT MODULATE MSCS FUNCTION AND AFFECT ENDOGENOUS BONE REGENRATION VIA THE MITOCHONDRIAL/OXIDATIVE STRESS PATHWAY

Sven Geissler[1], Martin Textor[1], Katharina Schmidt-Bleek[1], Oliver Klein[2], Mario Thiele[1], Dorit Jaobi[1], Anke Dienelt[1], Grit Kasper[1], Patrick Strube[3], Georg N. Duda[1]

[A]Julius Wolff Institute & Berlin-Brandenburg Center for Regenerative Therpies, [B]Institute for Medical and Human Genetics & Berlin-Brandenburg Center for Regenerative Therapies, [C]Julius Wolff Institute & Center for Musculoskeletal Surgery, [1]Charite Universitaetsmedizin Berlin, Berlin, Germany

Introduction: There is abundant evidence suggesting a decline in regenerative potential of tissues and organs with increasing age. Even tissues capable of complete regeneration, such as bone, show an age-related reduction in their healing capacity. We hypothesized that this decline is not only associated with the age-related reduction in cell quantity and function but also results from changes in the systemic environment. Thus, we investigated the influence of serum from young and aged Sprague-Dawley rats on MSC at the cellular and molecular level.
Results: We could demonstrate that age-related changes in the systemic environment negatively affected MSC's survival and differentiation. In particular, cultivation of MSCs in serum of aged animals enhanced their apoptosis rate, reduced their proliferation potential, compromised their osteogenic differentiation ability, and promoted their differentiation into the adipogenic lineage. Results of subsequent proteome (2DE MS/MS and Western Blot analysis) and cellular analysis identified enhanced intracellular oxidative stress as the underlying cause for the compromised MSC function in response to the age-altered systemic environment. Serum from aged animals not only changed the expression of proteins related to mitochondria, unfolded protein binding and stress response, it also significantly enhanced intracellular ROS production and lead to the accumulation of oxidatively damaged proteins. Conversely, reduction of oxidative stress levels by antioxidant supplementation in-vitro or by oral administration in-vivo markedly improved MSC function and bone regeneration, respectively. In aged animals, the systemic antioxidant treatment significantly improved the mineralization, the microstructure, and the mechanical properties of the regenerated bone tissue.
Discussion/Conclusion: In summary, we propose that the systemic environment crucially contributes to the age-related decline in bone regeneration by increasing intracellular oxidative stress levels, hence compromising viability and function of mesenchymal (progenitor) cells. Our results are in line with other studies demonstrating that muscle and liver progenitor cells of aged mice can be rejuvenated in-vivo & in-vitro by exposure to a young systemic

milieu. Therefore, we provide evidence that extrinsic aging has a higher impact on the function of MSCs and other (mesenchymal) cell types than intrinsic aging. We conclude that, especially in elderly patients, novel therapeutic approaches for the improvement of endogenous (bone) regeneration should focus on ROS protection of mesenchymal cells at the site of injury, rather than simply on cell differentiation.

T-3058

DECIDUALIZATION OF FETAL BONE MARROW DERIVED MESENCHYMAL STEM CELLS (BMMSCS) IS GENDER DEPENDENT

Chin Wen Tan[1], Yie Hou Lee[1], Heng Hao Tan[2], Mahesh Choolani[3], Linda Griffith[4], Jerry Chan[5]

[1]BioSystems and Micromechanics (BioSyM) IRG, Singapore- MIT Alliance for Research and Technology (SMART), Singapore, Singapore, [2]Department of Reproductive Medicine, KK Women & Children Hospital, Singapore, Singapore, [3]Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore, [4]Department of Biological & Mechanical Engineering, Massachusetts Institute of Technology (MIT), Boston, MA, United States, [5]Cancer & Stem Cell Biology Program, Duke NUS Graduate Medical School, Singapore, Singapore

Introduction: Gender differences secondary to the sex hormones administration have contributed to different cell morphology and phenotype. Treatment with estrogen could increase MSCs proliferation more profoundly in female MSCs, and that in general female MSCs generates higher levels of VEGF, lesser IL-6 and TNF-α and undergo less apoptotsis in response to hypoxia and lipopolysaccharides than those from male subjects. Decidualization (stromal cell differentiation) is important in the process of menstruation and pregnancy. Studies have shown that BMMSCs are similar to endometrial stromal cells (ESCs) in terms of the surface cell markers and the decidualization capacity. What is not known however, is whether BMMSCs of different sex would exhibit different properties when being stimulated by sex hormones. Here we investigated the difference in prolactin secretion between fetal BMMSCs (fBMMSCs) and ESCs in response to decidualization stimulant. The effect of sex dimorphism in these fBMMSCs during prenatal development is discussed.
Materials and Methods: Six fBMMSCs samples having a gestational age between 13 to 19 weeks were chosen for this pilot study. Their respective gender were identified via FlashFISH. fBMMSCs were decidualized in typical MSCs culture media (DMEM) or ESCs culture media (DMEM-F12) supplemented with progesterone, estrogen, cyclic adenosine monophosphate (cAMP) or prostaglandin (PGE2). Charcoal- stripped or normal fetal bovine serum were supplemented at either 2% or 10% to the respective condition. Subsequent conditioned medium were collected for ELISA assay for prolactin (PRL) concentration.
Results: Stimulated by a combination of cAMP and PGE2 with low level charcoal- stripped serum, female fBMMSCs could secrete more prolactin than male donors at day 14 (1.104 vs 0.571 ug/106 cells/day; p = 0.00353) and day 21 (2.717 vs 1.339 ug/106 cells/day; p=0.00026) of induction, although the decidualization capacity was less profound as compared to ESCs.
Conclusions: Sexual dimorphism may exist in fBMMSCs when being decidualized, and these findings would help explain the role of BMMSCs during prenatal development and sex- linked diseases, as well as possible linkage to gynecological diseases such as endometriosis.

T-3061

THE Gαs-PKA AXIS MAINTAINS A BALANCE BETWEEN WNT/β-CATENIN AND HEDGEHOG SIGNALING PATHWAYS TO SPATIALLY RESTRICT MESENCHYMAL STEM CELL DIFFERENTIATION TO PREVENT ECTOPIC BONE

Deepti Malhotra[1], Jean Regard[2], Jelena Gvozdenovic-Jeremic[1], Min Chen[3], Lee S. Weinstein[3], Jianming Lu[4], Eileen M. Shore[5], Frederick S. Kaplan[5], Yingzi Yang[1]

[1]GDRB, NHGRI, NIH, Bethesda, MD, United States, [2]NIBR, Novartis, Boston, MA, United States, [3]NIDDK, NIH, Bethesda, MD, United States, [4]CODEX biosolutions, Bethesda, MD, United States, [5]Department of Orthopaedic Surgery, University of Pennsylvania, Philadelphia, PA, United States

**Bone formation is exquisitely controlled in space and time. Heterotopic ossification (HO), the pathologic formation of extra-skeletal bone, occurs as a common complication of trauma or in rare genetic disorders and can be disabling and lethal; however, the molecular mechanisms that spatially restrict bone formation are largely unknown. Here we identify ectopic Hedgehog (Hh) signaling as a seminal cause of HO in mouse models of the human disease progressive osseous heteroplasia (POH), which results from null mutations of *GNAS* encoding G_S. While, previous work from our lab had demonstrated an activating mutation in G_S to potentiate Wnt/β-catenin signaling strength towards abnormal skeletogenesis in human disease, Fibrous Dysplasia (FD). We show that inactivation of G_S decreases PKA activity, and consequently stimulate Hedgehog (Hh) signaling by activating Gli2 and inhibiting Gli3 repressor formation. At the same time, we observe a decline in Wnt/β-catenin targets, which accompany an uninhibited osteogenic differentiation from spatially inappropriate mesenchymal progenitors. Thus, we establish G_S signaling as a central hub that balances appropriate Wnt/β-catenin and Hh signaling strengths towards normal bone formation, while inhibiting aberrant mesenchymal stem cell differentiation. Moreover, in POH patient samples, Hh signaling is upregulated in ectopic osteoblasts; and ectopic activation of Hh signaling is sufficient to induce HO, while genetic inhibition of Hh signaling blocks HO in animal models. These findings identify G_S-PKA-Hh signaling as a centrally important regulatory axis that regulates
mesenchymal stem cell fate choices under homeostasis and disease, and suggest that the Hh pathway is a promising therapeutic target for HO.**

T-3062

RAP1 ACTIVATED MESENCHYMAL STEM CELLS TRANSPLANTED IN INFARCTED MYOCARDIUM IMPROVE HEART FUNCTION

Asmat Salim, Irfan Khan, Muhammad Aleem Akhter, Anwar Ali, Nadia Naeem, Hana'a Iqbal, Nurul Kabir
Dr. Panjwani Center for Molecular Medicine and Drug Research, Karachi, Pakistan

Small G-proteins are involved in cardiovascular regulation in numerous ways which include the regulation of endothelial function, vascular smooth muscle cell contraction and proliferation, stem cell migration, and cardiac hypertrophy. Individual cells in their particular environment adhere to the extracellular matrix and their neighboring cells via integrin and cadherin containing complexes. The dynamics of these interactions regulate the formation and maintenance of complex tissue. Rap1, a member of the Ras superfamily of small GTP-binding proteins is an evolutionary conserved regulator of several basic cellular functions such as adhesion, polarity, differentiation and growth. Rap1a is integral to signaling pathways that regulate various aspects of different adhesion systems. In this study, mesenchymal stem cells (MSCs) and cardiomyocytes (CMs) were isolated from bone marrow and neonate heart respectively, cultured, and characterized by immunocytochemistry and flow cytometry. Rap GTPase was activated by 8-pCPT-2'-O-Me-cAMP in MSCs and CMs. Cell adhesion molecules (Timp3, I-cam1, V-cam1, Adamts) in normal and activated MSCs and CMs were analyzed with RT-PCR. It was observed that activation of Rap1 in CMs leads to up-regulation of cell adhesion molecules. Co-culture analysis of normal and activated MSCs and CMs showed that activated cells have higher fusion capability as compared to normal cells. For *in vivo* studies, myocardial infarction was produced through occlusion of the left anterior descending coronary artery in rats. The study was randomized for cell-therapy group (n-5) and control group (n-5). One million mesenchymal stem cells (normal, hypoxic, Rap 1 activated or both) were injected through intramyocardial injection into the infarcted area. Cardiac function was measured by using echocardiography pre-operatively and at 2-4 weeks after MSCs transplantation. The ejection fraction was improved in the injected cells group but not in the control group at 2-4 weeks. The exact mechanism remains to be determined, but our findings suggest that Rap1 improves cardiac function probably through better survival and adhesion of transplanted cells.

T-3063

TRAUMATIZED MUSCLE-DERIVED MULTIPOTENTIAL PROGENITOR CELLS ARE CANDIDATE CELLS RESPONSIBLE FOR HETEROTOPIC OSSIFICATION: REGULATION OF OSTEOGENESIS

Peter G. Alexander[1], Karen L. Clark[1], Anthony WM Cheng[1], Hang Lin[1], Youngmi Ji[2], Leon J. Nesti[2], Rocky S. Tuan[1]
[1]Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA, United States, [2]Clinical and Experimental Orthopaedics, NIH, Bethesda, MD, United States

Heterotopic ossification (HO), the formation of mature bone in the soft tissues, is a frequent complication following trauma. The pathogenesis of HO is not well understood, but is hypothesized to involve an inappropriate cellular response to the inflammatory environment and specific osteogenic signals therein. We have previously isolated and characterized a population of mesenchymal progenitor cells (MPCs) derived from human blast-traumatized muscle that may represent the cellular component of the disease. The MPCs express several markers common to bone marrow-derived mesenchymal stem cells (MSCs), including CD73, CD90, and CD105, and can undergo multilineage differentiation. The traumatized muscle environment contains elevated levels of cytokines associated with osteogenesis and fibrosis (i.e., BMP1 and TGFβ1), suggesting that inhibition of the BMP1/TGF-b1 pathway may be effective in inhibiting HO. In this study, we have characterized the cytokine profile of the traumatized muscle-derived MPCs, and test the ability of a BMPR1 inhibitor, dorsomorphin (DM), to block MPC osteogenesis in vitro using 2D and 3D cultures, as well as in an in vivo model of HO. The stem cell nature of the MPCs was shown using CFU assays, RT-PCR (to detect CD73, CD90, and CD105 expression), and multilineage differentiation assays (osteo-, chondro- and adipogenesis). RT-PCR array analysis revealed elevated expression of TGFβ3, BMP6 and 7 in these cells. Osteogenesis in 2D, 3D pellet, and collagen I (Col1) hydrogel culture (4 x 106 cells/ml) was induced with 50 ng/ml BMP2 with or without 2.5 mM DM. Osteogenesis was assessed by alizarin red staining in 2D cultures, and by RT-PCR and micro-CT in 3D cultures. Osteogenesis was observed in 2D, pellet, and Col1 hydrogel only with BMP2 treatment. In 2D MPC cultures, DM treatment significantly suppressed BMP2-induced osteogenesis. Histologically robust BMP2-induced osteogenesis was seen in both pellet and ColI hydrogel cultures, and significantly increased expression of osteogenic genes (Runx2, bone sialoprotein (BSP), Col1, alkaline phosphatase (ALP) and osteocalcin) was seen at week 5. At week 7, osteogenic gene expression by MPCs in ColI hydrogels was higher than in pellet culture (2- to 5-fold for Runx2, BSP and Col1). Micro-CT revealed that DM application to BMP2-stimulated pellet cultures reduced tissue calcification to control, unstimulated levels (>95% reduction), while calcification in BMP2 treated 3D Col1 constructs was reduced >40% by DM. These in vitro data suggest that DM is a promising therapeutic for HO. Ongoing studies use an immunodeficient mouse model to examine the efficacy of DM to reduce ectopic bone formation resulting from MPC injection with or without co-administration of BMP2. Results from these studies should shed light on HO pathogenesis and prevention/treatment.

T-3064

THE ROLE OF sFRP2 IN HUMAN MESENCHYMAL STEM CELLS UNDER OXIDATIVE STRESS CONDITION

Yaowalak U-pratya[1], Kanjana Pomduk[2], Pakpoom Kheolamai[5], Aungkura Supokawej[3], Sirikul Manochantr[5], Methichit Wattanapanitch[4], Surapol Issaragrisil[1]
[A]Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, [B]Department of Immunology, Faculty of Medicine Siriraj Hospital, [C]Department of Clinical Microscopy, Faculty of Medical Technology, [D]Siriraj Center of Excellence for Stem Cell Research, Faculty of Medicine Siriraj Hospital, [1]Mahidol University, Bangkok, Thailand, [2]Division of Cell Biology, Department of Pre-clinical Sciences, Faculty of Medicine, Thammasat University, Patumthani, Thailand

Human mesenchymal stem cells (hMSCs) have great promise for improving injured and degenerated tissues associated with several diseases. However, based on animal studies, the therapeutic potential
of hMSCs is limited due to the poor survivability of hMSCs after transplantation. This is, at least in part, due to the oxidative stress present in the microenvironment of the injured tissues. We investigated the improvement of the viability of hMSCs under oxidative stress conditions by overexpressing the *secreted frizzled-related protein-2 (sFRP2)* gene. sFRP2 is a member of the secreted frizzled-related protein (sFRP) family which binds to Wnt glycoproteins resulting in the modulation of Wnt signaling cascades that ultimately prevent the oxidative stress-induced apoptosis in several cell types. In this study, the whole coding sequences of sFRP2 was inserted into the expression vector,

pLenti 6.2/V5-DEST, before transduction into hMSCs by lentivirus. The transduced hMSCs (sFRP2-hMSCs) were then cultured and treated with 0.75 mM H_2O_2 for 5 hours to simulate the oxidative stress conditions *in vitro*. Our results showed that the apoptotic rates of sFRP2-hMSCs under H_2O_2-induced
oxidative stress condition, as determined by flow cytometry, were significantly reduced in comparison to those of non-transduced hMSCs cultured under the same condition. In conclusion, sFRP2 gene can augment the survivability of hMSCs under oxidative stress condition and may be used to improve the viability and enhance engraftment of hMSCs after transplantation.

T-3065

DIFFERENTIALLY EXPRESSED GENES ASSOCIATED TO HIGHER OSTEOGENIC POTENTIAL OF HUMAN MESENCHYMAL STEM CELLS

Roberto Fanganiello, Felipe A.A. Ishiy, Daniele Yumi, Luciane P. Capelo, Maria Rita S. Passos-Bueno
Genetics and Evolutionary Biology, University of Sao Paulo, Sao Paulo, Brazil

Stem cells from human exfoliated deciduous teeth (SHED) and human adipose tissue-derived stem cells (hASCs) are interesting subtypes of adult mesenchymal stem cells (MSCs) used in bone bioengineering studies due to their osteogenic properties and minimally invasive process of isolation. There are many in vitro and in vivo studies exploring the use of SHED and hASC as an alternative to the broadly used bone marrow stem cells (BMSCs) for bone tissue engineering. In a previous work we showed that SHED are more osteogenic than hASCs under the same in vitro and in vivo controlled conditions. Here, we aimed to identify alterations in gene expression associated to this difference. Stem cell populations were isolated from dental pulp, adipose tissue and bone marrow from six independent healthy donors (two for each group) and induced to in vitro osteogenesis. Confirming our previous results, SHED and BMSCs showed increase in ALP and RUNX2 gene expression during early osteogenesis, higher alkaline phosphatase activity ($p \leq 0.05$) after 9 days and increase in matrix mineralization ($p \leq 0.001$) after 21 days. Gene expression microarray assays (Affymetrix GeneChip Human Gene 1.0 ST) were performed at three time points of early in vitro osteogenesis: immediately before induction (day 0) and at days 4 and 6 of osteoinduction. Raw microarray data were normalized through robust multichip average and interquartile range (IQR<1) and batch effect corrections (ComBat) were applied. Multiclass significance analysis for microarrays (SAM, $p \leq 0.05$) adjusted by false discovery rate correction was used to detect the differentially expressed genes (DEGs) associated to each stem cell group. Significant changes in gene expression profile along the early in vitro osteogenic differentiation were detected in 198 transcripts for BMSCs, 34 transcripts for SHED and 87 transcripts for hASCs. Through Ingenuity Pathway Analysis, we found enrichment in interleukins 17A and LXR/RXR pathways, both previously associated to osteogenesis. Eleven DEGs were common between BMSCs and SHED and seven of them have already been associated to osteogenesis (ABCA6, AMIGO2, OMD, TIMP4, METTL7A, APOD and TMTC1). These observations not only confirm but also elucidate the molecular events that may lead to an enhanced osteogenic potential in a MSCs. Next, our analysis revealed that before osteoinduction, IGF2 and ITGA8 were the top upregulated transcripts in SHED and BMSCs when compared to hASCs, and that IGF2 remained upregulated in these MSCs until day 6. IGF2 is well known for its role in the regulation of bone metabolic processes and ITGA8 has been shown to interact with osteopontin during the process of bone mineralization. We further validated IGF2 upregulation through quantitative real-time PCR in other 10 SHED (average of 32.2-fold, $p \leq 0.01$) compared to 10 hASCs samples. These results suggest that assessment of IGF2 expression may be used to predict the in vitro osteogenic potential of MSCs. Altogether, our study identified key pathways that are linked to osteogenesis and we propose that IGF2 is an interesting potential biomarker to select stem cell populations for bone tissue engineering.

T-3066

PREDICTIVE MODELING AND BIOMECHANICAL MICROENGINEERING OF HUMAN MESENCHYMAL STEM CELLS: A HIGH-CONTENT SCREENING PLATFORM TO ELUCIDATE AND ENHANCE LINEAGE SPECIFIC DIFFERENTIATION

Amit Paul, David Franz, Sumaira Yahya, Shan Sun, Michael Cho
Bioengineering, University of Illinois at Chicago, Chicago, IL, United States

The cytoskeletal structures and arrangements in stem cells undergoing differentiation are dramatically altered, and these alterations vary by lineage. The complexity of events associated with the transformation of these precursor cells leaves many questions unanswered about morphological, structural, proteomic, and functional changes in differentiating stem cells. A thorough understanding of stem cell behavior, both experimentally and computationally, would allow development of more effective approaches to the expansion of stem cells in vitro and for the regulation of their commitment to a specific phenotype. We therefore investigated human mesenchymal stem cell (MSC) differentiation, as MSCs exhibit diverse multipotency. Through traditional in vitro biochemical approaches, MSCs were differentiated into adipocytes, osteoblasts, chondrocytes, cardiomyocytes, and neurons. Lineage-specific fluorescent labels were carefully selected, and immunocytochemistry was performed via quantitative 2D/3D fluorescence microscopy. MSCs were also visualized label-free via FTIR spectroscopic imaging. For all of the cellular structures imaged, over 100 unique image-based measurements were taken at crucial differentiation time points. Additionally, the dynamic lineage-dependent biomechanical changes were measured using atomic force microscopy, laser optical tweezers, and electrical activity mapping. With the abundance of quantitative experimental data, we developed a sophisticated high-content screening (HCS) process that automatically analyzes & predicts the mechanical transformations which are potentially required to initiate the onset of stem cell differentiation into tissue-specific phenotypes. This novel HCS system was integrated with laser capture microdissection (LCM) to automatically identify and capture differentiating stem cells in a selective manner for gene expression profiling. With the assistance of this advanced computational model of stem cell differentiation, many aspects of MSC differentiation were thoroughly elucidated. Perhaps most significant was a consistent pattern observed in all types of MSC differentiation: the cytoskeleton remodeled significantly before lineage-specific cellular changes occurred. This strongly suggests that cellular mechanical transformations are a precursor to stem cell differentiation. Based on these detailed findings, MSCs were directed into specified phenotypes by forcing the stem cells into 3D biomechanical geometries (these geometries were elucidated from the HCS). We used modern microfabrication techniques to engineer single-cell/multicellular microenvironments that were biomimetic (mechanically, geometrically, and biochemically) to the desired lineage. The MSCs were forced into the microenvironments. Once the MSCs fully differentiated, they exhibited a higher degree of phenotypic similarity to the target lineage compared to MSCs differentiated using traditional in vitro methods. The biomimetic microenvironments significantly enhanced MSC differentiation, further demonstrating the vast role cellular mechanics play in stem cell differentiation. This innovative platform of stem cell differentiation (automated HCS, automated LCM, and biomimetic microengineering) will soon be extended to other types of stem cells (e.g., induced pluripotent stem cells) and can eventually be adapted to tailor patient-specific treatments for various stem cell therapies.

T-3067

HYPOXIA ATTENUATES HYPERTROPHY IN CHONDROCYTES DERIVED FROM MESENCHYMAL STEM CELLS BY ZFP521-MEDIATED DOWNREGULATION OF RUNX2

David Browe[1], Stephen J. Elliman[2], Cynthia Coleman[1], Frank Barry[1]

[A]Regenerative Medicine Institute, [B]Orbsen Therapeutics Ltd., [1]NUI Galway, Galway, Ireland

Mesenchymal stem cells (MSCs) represent a cellular strategy for repairing osteochondral defects or tissue damaged by osteoarthritis. However, human MSC-derived chondrocytes undergo hypertrophic differentiation, forming mineralized cartilage and bone via endochondral ossification rather than true hyaline cartilage. Strategies that limit hypertrophy may improve the quality of MSC-derived cartilage and lead to more effective tissue repair.
Artifacts from the in vitro culture and differentiation of MSCs may stimulate their hypertrophic differentiation. Cartilage formation occurs in vivo at low oxygen tensions (<2% oxygen), whereas in vitro protocols for chondrogenic differentiation of MSCs routinely rely on higher levels of oxygen (typically 19%). This increase in oxygen saturation stimulates prolyl hydroxylases (PHD), which require oxygen and iron as co-factors to hydroxylate hypoxia-induced factors (HIF1α and HIF2α), resulting in hypertrophic activity. We demonstrated that MSC-micromass pellets cultured at low oxygen tension deposit an extracellular matrix with significantly increased glycosaminoglycan (3-fold increase) and collagen II deposition. Several important markers associated with hypertrophic differentiation were also examined and we observed a reduction in collagen X and Runt-related transcription factor 2 (RUNX2) protein

deposition in MSC-micromass pellets cultured at low oxygen tension. A 2-fold decrease in alkaline phosphatase activity from MSC-micromass pellet conditioned media differentiated at low oxygen tension was also demonstrated.
Previous studies have demonstrated that addition of parathyroid hormone-related protein (PTHrP) 1-34 can effectively attenuate hypertrophic markers. We have demonstrated that physiological hypoxia can increase PTHrP secretion by MSCs undergoing chondrogenic differentiation, a 2.5-fold increase in PTHrP was detected in conditioned media at days 14 and 21 of differentiation when compared to normoxic controls. Zinc finger protein 521 (Zfp521) is a transcriptional co-regulator that is a key effector of PTHrP signalling in growth plate chondrocytes. PTHrP has been observed to increase expression of Zfp521 in chondrocytes and in several chondrogenic cell lines, this increased expression of Zfp521 results in a repression of RUNX2 activity. We hypothesize that manipulation of oxygen related pathways attenuates hypertrophy in cartilage formed from MSCs via an increase in PTHrP secretion, Zfp521 mediated repression of RUNX2 and associated repression of downstream markers of hypertrophy such as collagen X, Indian hedgehog, and matrix metallopeptidase 13. Therefore the phenotype of articular cartilage derived from MSCs can be controlled by the action of PTHrP and PHD inhibitors. This represents a novel cartilage repair strategy involving controlled chondrogenic commitment of MSCs.

T-3068

EPIGENETIC DEDIFFERENTIATION RETAINS THE IMMUNOPRIVELEGE OF HUMAN AMNIOTIC MESENCHYMAL STEM CELLS

Il Suk Sohn, Yuka Matsuura, Paul J. Kim, Rajesh Dash, Phillip C. Yang
Department of Medicine, Stanford University School of Medicine, Stanford, CA, United States

Background
The human amniotic mesenchymal stem cells (hAMSCs) demonstrate immunomodulatory property. High native expression of CD59, human leukocyte antigen (HLA)-G, HLA-E, intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM) -1 with the absence of HLA-DR enables xenogeneic survival of hAMSCs. Both HLA-G and HLA-E are non-classical MHC class I molecule and may have immunomodulatory function and both genes are transcribed in amnion cells. ICAM-1 and VCAM-1 are known to be critical for mesenchymal stem cell -mediated immunosuppression. However, this unique immunoprivelege is diminished following multiple in vitro cell culture passages. Epigenetic reprogramming by valproic acid (VPA), a histone deacetylase inhibitor, dedifferentiates the human amniotic mesenchymal stem cells (hAMSCs). VPA induces intermediate pluripotency state to provide an epigenetically unstable environment and allows re-expression of early immunophenotype in hAMSCs. In this study, we examined the re-emergence of immunoprivelege in hAMSCs following epigenetic reprogramming of hAMSCs with VPA into a more developmentally naïve state.
Methods
The hAMSCs were enzymatically digested from human placenta amniotic membrane and cultured in hAMSC media. On reaching 80% confluence, the media were changed to human embryonic stem cell (hESC) culture condition with 1.0, 3.0 and 6.0mM of VPA (Sigma-Aldrich, Missouri) for 5 days. Flow cytometry and real time PCR were performed before and after VPA treatment to evaluate for re-emergence of cell surface marker and gene expression of immunoprivelege.
Results
The hAMSCs underwent robust non-integrative epigenetic reprogramming using VPA in hESC culture condition to generate a dedifferentiated, partially reprogrammed population of hAMSCs, marked by the emergence of 50% Tra1-60 positive cells and significant up-regulation of Nanog, Brachyury and GATA4 genes (5-, 10-, and 10-fold, respectively). At low concentration (1mM) of VPA for 5 days, hAMSCs showed marked increase in surface markers of immunogenic HLA. The HLA-A, B, C, and DR increased by 24- and 20-fold, respectively. Similarly, immunosuppressive HLA-G and HLA-E increased by 22- and 30-fold respectively. The immunomodulatory adhesion molecules, ICAM-1 increased by 6 fold and VCAM-1 increased by 32 fold. Following 5-day treatment with 1mM VPA, additional increase in gene expression for HLA-A (17-fold), HLA-B (12-fold), HLA-C (10-fold), HLA-DR (23-fold), HLA-G(4-fold), HLA-E (17-fold), VCAM-1 (10-fold), and ICAM-1 (6-fold) was noted. In addition, CD 59, an inhibitor of complement-

mediated membrane attack complex, demonstrated robust gene expression and surface marker (96% positivity) after VPA.

Conclusion

VPA modulates the progress of immunoprivelege of hAMSCs in vitro. These unique immunomodulatory cells are readily reprogrammed with VPA and may enable rapid translation of regenerative medicine. Further studies to assess their in vivo survival are underway.

T-3071

LONG TERM INTEGRATION OF ADIPOSE DERIVED MESENCHYMAL STEM CELLS IN LYMPH NODE

Torunn Rønningen, Philippe Collas, **Jan Ø. Moskaug**
University of Oslo, Oslo, Norway

Mesenchymal stem cells (MSCs) are able to differentiate to several lineages, including lymph node stromal cells. We show that luminescently and fluorescently labeled MSCs derived from adipose tissue in adult mice are detected at discrete locations after injection into the peritoneal cavity. In vivo imaging and end point dissection followed by ex vivo luminescence imaging several months after i.p. injection revealed that cells were stably integrated in lymph nodes adjacent to organs and tissues in the abdominal cavity. Our results strongly support the notion that not only bone marrow derived mesenchymal stem cells, but also those derived from adipose tissue are able to migrate and settle down in lymphoid tissue, where they may exert immuno and inflammation regulatory functions.

T-3072

RNA HELICASE A, AN MRNA TARGET AND PROTEIN PARTNER OF LIN28 IN HESCS, IS INDISPENSABLE FOR LIN28---MEDIATED TRANSLATION

LIN GENG
Yale Stem Cell Center and Department of Cell Biology, Yale University School of Medicine, New Haven, NEW HAVEN, CT, United States

RNA helicase A (RHA) and Lin28 are RNA binding proteins involved in translational regulation of gene expression. In human embryonic stem cells (hESCs), LIN28 binds to
mRNAs of pluripotent gene *OCT4* and several metabolically related genes and positively
regulates their translation. However, the mechanisms through which LIN28 regulates
pluripotent and metabolic processes are still unclear. We previously demonstrated that
RHA interacts with LIN28 via protein---protein interaction. Here we demonstrated that RHA and LIN28 collaborate with each other to up---regulate translation of LIN28---targeted mRNAs. Interestingly, RHA mRNA itself is a target of LIN28 and is translationally regulated by LIN28. Therefore, LIN28 facilitates a positive Loop of RHA and active gene translation for those genes recoginized by LIN28. Furthermore, LIN28 promotes the cytoplasmic localization of RHA and helps to recruit RHA onto polysomes to facilitate translation. Such a mechanism may also be used for LIN28 to regulate the translation of metabolicgenes.

T-3073

AGE RELATED CHANGES OF KIDNEY MESENCHYMAL STROMAL CELLS PROMOTE MONOCYTE INFLAMMATORY RESPONSES

Victorine Douin-Echinard, Sidy M. Casse, Pauline Decaunes, Caroline Ceccaldi, Joffrey Pozzo, Nathalie Pizzinat, Angelo Parini, Daniel Cussac
Cardiac remodelling : physiopathology and new therapeutics, I2MC Inserm U1048, Toulouse, France

Mesenchymal stromal cells (MSCs) have been characterized in bone marrow and adipose tissues but also in most solid organs of human and rodents including kidney. Beside their capacity to differentiate in cells of the meso-

dermal lineage, they play a central role in tissue regeneration, wound repair and maintenance of tissue homeostasis through their paracrine activity. We hypothesized that ageing may modify the paracrine activity of MSCs and ultimately affect age-related changes of kidney stromal microenvironment by immune-modulating signals. We found that sca-1^+ $CD31^-$ $CD45^-$ MSCs absolute number per mg of kidney was decreased by 2-fold in 24 months aged mice compared to 3 month old. On the other hand, kidney MSCs (k-MSCs) subpopulation frequencies remained comparable for CD29, CD24, CD73 and CD105 surface expression. After *in vitro* selection by adhesion and expansion, positive sca-1^+ $CD31^-$ $CD45^-$ MSCs from old mice depicted increase expression in the replicative senescence marker p57 and in ß-galactosidase activity. Major modifications related to senescence concerned the increased expression of chemokines and growth factors such as CCL2 and IGF-1. In order to evaluate the impact of this senescence-associated secretory phenotype on monocyte function we performed co-cultures with monocytes isolated from bone marrow of young donors. We demonstrated that k-MSCs potentiated the secretion and the frequency of TNF-α and IL-6 double positive $Ly6C^{high}$ $CX3CR1^{int}$ inflammatory monocytes in response to TLR4 stimulation. Furthermore, aged k-MSCs were more potent than young k-MSCs to promote IL-6 secretion in the co-cultures. These effects were dependent on cell-cell interactions suggesting a different integration of TLR4 dependant signalling in aged k-MSCs. Together these results showed that age-related changes of k-MSCs promoted a kidney stromal microenvironment which favoured inflammatory monocyte responsiveness and could be deleterious and promote kidney damage in the elderly.

T-3074

BYSTANDER EFFECT OBSERVED FOR THE GAMMA-IRRADIATED MESENCHYMAL STEM CELLS FROM BONE MARROW OF RATS UNDERGONE THE CARCINOGENIC ACTION OF 1,2-DIMETHYLHYDRAZINE

Anatoly Konoplyannikov[1], Sagida Kalsina[1], Veronika Revkova[1], Mikhail Konoplyannikov[2]

[1]Medical Radiological Research Center, Obninsk, Russian Federation, [2]Federal Research Clinical Center, Moscow, Russian Federation

The carcinogenic action onto adult stem cells may lead to the changes in their properties which are similar to those observed for cancer stem cells, i.e. increased cell resistance to radiation. We have demonstrated this effect earlier for the carcinogenic action of 1,2-dimethylhydrazine onto hematopoietic stem cells and intestinal stem cells of mice, the irradiation of the animals being conducted one day after the carcinogen administration. The survival of these two types of adult stem cells after the treatment with different dosages of Co^{60} γ-rays (registered by the in vivo spleen endocolony and intestinal “microcolony” tests) was statistically significantly enhanced as compared to the survival of stem cells of intact animals not undergone the carcinogen action. We also reproduced this effect in the experiments on the post-radiation survival of mesenchymal stem cells (MSC) from bone marrow of Wistar rats, in which the cells survival was estimated by the production of cell clones in the cell culture in vitro. However, in this latter case we also revealed a so-called “bystander effect” which manifested itself in the fact that the post-radiation survival of irradiated MSC enhanced with the increase in the cell culture density in vitro. This finding testifies that there exists a certain local interaction between irradiated MSC suffered the carcinogen action, which results in the regeneration of the lethally damaged cells’ ability to the prolonged proliferation with formation of cell clones. The nature of such interaction remains unknown, it is probable that paracrine agents participate in its realization, which account for the MSC’s therapeutic effect. We have no doubt that studying those mechanisms is of importance, in hope one can find the inhibitors of the “bystander-effect” and can boost the efficiency of the radiation therapy towards resistant forms of malignant neoplasms.

T-3075

OXYTOCIN AS A NOVEL INDUCER FOR DIFFERENTIATION OF HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS INTO CARDIOMYOCYTE LIKE CELLS

Sujata Mohanty[1], Anupama Kakkar[1], Anil Kumar[1], Sushmita Bose[1], Himi Singh[1], Balram Bhagava[2], Balram Airan[3]

[A]Stem Cell Facility, [B]Cardiology, [C]Cardiothoracic & Vascular Surgery, [1]AIIMS, New Delhi, New Delhi, India

Stem cells are now being explored as a potential and reliable treatment option for various degenerative diseases including cardiovascular diseases (CVDs). Mesenchymal Stem Cells (MSCs) are considered as one of the best candidates for regeneration because of their multipotency, immunological naivety and absence of ethical issues. 5-Azacytidine is the most widely used inducer for the cardiomyogenic differentiation of stem cells both Embryonic Stem Cells and Adult stem Cells. However, it has demethylating and cytotoxic effect on cells; which limits its use to derive cardiomyocytes for future transplantation use. Recent reports including ours, has suggested TGFβ1 as an alternate inducer. However, its differentiation efficiency is less than that of 5-Azacytidine. The present study was initiated to explore the potential of other cardiomyogenic inducers. We compared the potential of Oxytocin and TGFβ1 with 5-Azacytidine, for the efficient generation of cardiomyocytes from human bone marrow derived MSC (hBM-MSC).

Study was initiated after the approval from Institute Ethics Committee & Stem Cell Ethics Committee. Cryopreserved hBM-MSCs were used in this study (n=3). Cells were expanded in LG-DMEM supplemented with 10% FBS at 37ºC/5%CO2. After establishment of culture, cells were expanded and characterized. All the experiments were performed using 3rd passage cells after their characterisation. Morphologically, they were adherent, spindle shaped and grew in monolayer. They were checked for the expression of vimentin and Fibroblast Specific Protein (FSP) by Immunofluorescence (IF) assay; Surface markers -CD105, CD90, CD29, CD73, HLA CIass I & Class II and CD 34/45 by Flowcytometry. After characterization, the cells were differentiated in vitro into cardiac like cells using 3 induction protocols. LG-DMEM supplemented with 10% FBS was common to all three protocols. Apart from this, Protocol I used 5-Azacytidine, 6uM, Protocol II used TGFβ1, 10ng/ml, and Protocol III used Oxytocin, 100nM as inducers. Morphological changes were observed and documented. Differentiated cells were characterized for cardiac related markers : Myosin light chain -2v (Mlc-2v), Cardiac Actin (CA), Connexin 43 (Cx43), GATA4, Cardiac Troponin I (cTnI) by Reverse Transcriptase-PCR (RT-PCR), cTnI and Mlc-2v by Immunofluorescence (IF), cTnI and Mlc-2v by Flow cytometry and Mlc-2v by quantitative RT-PCR (qRT-PCR).

hBM-MSCs were found to be positive for vimentin, CD105, CD90, CD29, CD73, HLA CIass I and negative for FSP, HLA Class II and CD34/45. Differentiation studies revealed the expression of Mlc-2v, CA, Cx43, GATA4, cTnI by RT-PCR and Mlc-2v and cTnI by IF in all the three treatment groups. Expression of cardiac related markers was almost equivalent in cells treated with Protocol I & Protocol III (Protocol I-Mlc-2v=21.66%, cTnI=22% & Protocol III (Mlc-2v=25%, cTnI=22%). followed by Protocol II (Mlc-2v=13.33%, cTnI=15.3%) by Flow Cytometer analysis (Difference was nonsignificant P value > 0.05). This was further confirmed by qPCR for cardiac specific gene Mlc-2v in which cells treated with Protocol I showed 3 fold expression, followed by Protocol III with 2.8 fold expression and least with Protocol II ie.1.7 fold expression.

These Initial studies reveal that Oxytocin, a natural hormone present in our body which has the ability to induce the hBM-MSC into Cardiomyocytes like cells. Its differentiation ability is equivalent to 5-Aza, without any cytotoxic and demethylating effect on cells.

T-3076

DYNAMIC BEHAVIOR OF MESENCHYMAL STEM CELLS IN A CARDIAC MICROENVIRONMENT: A HIGH RESOLUTION TIME-LAPSE IMAGING STUDY

Payal V. Pradhan[1], Maria C. Collins[1], Lauren Coltraine[1], **Barbara J. Muller-Borer**[2]

[A]Cardiovascular Sciences, [B]Engineering, [1]East Carolina University, Greenville, NC, United States

Human mesenchymal stem cells (hMSCs), when cultured in an *in vitro* cardiac microenvironment, establish cell-cell communications and express cardiomyocyte specific genes and proteins as early as 24 hours after co-culture. However, hMSC differentiation to a cardiac-like phenotype is observed only after days in culture. The focus of this investigation was to quantitate dynamic hMSC behavior to elucidate the time course of hMSC differentiation to a cardiac-like phenotype. **Methods**: Neonatal rat ventricular cardiomyocytes were isolated and grown in monolayers in multiwell tissue culture plates. HMSCs expressing mitochondrial dsRed were added to the cardiomyocyte cultures at a ratio of 1:10 (hMSCs:myocytes). Time-lapse recordings were performed on a Zeiss Axio Observer inver-

ted microscope with full incubation (37° C, 5% CO_2). Phase contrast and fluorescence images were acquired with a 20x/0.8 NA Plan Achroplan objective, and AxioCam MRM CCD camera. Regions of interest (ROI), 440 x 333 µm, were identified (one per well). Images of each ROI were acquired every 10 minutes over a 14hr period at 24 - 48 hrs (Group1), 7 days (Group 2) and 14 days (Group 3) after co-culture. The co-cultures were maintained in a cell culture incubator between imaging sessions and the protocol was repeated in 3 separate co-cultures. Spatio-temporal characteristics of the hMSCs were evaluated with ImagePro cell tracking software. **Results:** For each Group, ROIs with at least 4 hMSCs were identified in the initial image. However, an average of 2 hMSCs were tracked per ROI as only hMSCs that remained within the ROI for the duration of the imaging sequence were evaluated. With increased time in co-culture the average speed of hMSCs significantly decreased ($p \leq 0.05$). The speed of Group 1 hMSCs was faster than hMSCs in Group 2 and Group 3 (22 ± 1 vs. 16 ± 1 µm/hr and 22 ± 1 vs. 17 ± 1 µm/hr respectively). There was no difference in hMSC speed in Group 2 vs. Group 3 hMSCs. In addition, Group 1 hMSCs traveled significantly farther ($p \leq 0.05$) in the first 4 hours of observation compared to hMSCs in Group 2 and Group 3 during the same time period (88 ± 4 vs. 73 ± 4 µm and 88 ± 4 vs. 80 ± 5 µm respectively) **Conclusions:** Using an *in vitro* co-culture model we successfully imaged and tracked hMSC dynamics in a cardiac microenvironment. HMSC mobility during the 14 day observation period decreased, correlating with the observed time for hMSC differentiation to a cardiac-like phenotype. Understanding spatio-temporal hMSC behavior is important for developing successful strategies for cell transplantation therapies and understanding molecular mechanisms controlling hMSC survival, engraftment, differentiation and functional integration with the host tissue.

T-3077

POST-TRANSCRIPTIONAL UPREGULATION OF SOX9 AND AGGRECAN FACILITATED BY MICRORNA-140 DURING CHONDROGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS

Tommy A. Karlsen[1], Rune B. Jakobsen[1], Tarjei S. Mikkelsen[2], Jan E. Brinchmann[1]

[1]Norwegian Center for Stem Cell Research, Oslo University Hospital, Oslo, Norway, [2]Harvard Stem Cell Institute and Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA and Broad Institute Harvard-MIT, Cambridge, MA, USA, Boston, MA, United States

Introduction

MicroRNAs (miRNAs) are small endogenous RNA molecules that regulate gene expression in a sequence-dependent manner and are thought to be involved in most biological processes, including stem cell differentiation. MiRNAs interact with complementary mRNA molecules leading to either degradation of the mRNA or translational repression. Recently, miRNAs have also been shown to enhance gene expression. In some situations, this may occur through activation rather than repression of translation. In other situations miRNAs may bind to promoters with complementary sequences to induce, or repress, transcription. One particular miRNA, miR-140, has been shown to be cartilage specific. Indeed, miR-140 is important for cartilage development and homeostasis as miR-140-knockout mice have impaired endochondral bone formation and an OA-like pathology, while transgenic mice overexpressing miR-140 are resistant to antigen-induced arthritis. MiR-140 expression is positively regulated by the master regulator of chondrogenesis SOX9. The molecular mechanisms for the profound effect exerted by miR-140 on cartilage development are not fully known.

Objective

The objective of this study was:

- to compare the global miRNA expression profiles during chondrogenic differentiation of MSCs with that found in native and in vitro dedifferentiated articular chondrocytes
- to investigate the role of miRNAs that showed a reciprocal relationship during dedifferentiation of ACs and chondrogenic differentiation of MSCs
- to identify new miRNA targets

Materials and methods

ACs were allowed to dedifferentiate in monolayer cultures and MSCs were differentiated to chondrocytes in alginate gels. At different time points samples were harvested for global miRNA profiling. Transient transfection and

lentiviral transduction of miR-140-5p and miR-140-3p mimics and inhibitors were performed to investigate the role of these miRNA in cartilage development. Transfected and transduced cells were analyzed with microarrays, qPCR, western blot and glycosaminoglycan secretion analysis. Luciferase reporter assay was used to verify targets of miR-140-5p.<br /
Results/conclusion
MiR-140-5p and miR-140-3p were among the most highly expressed miRNAs in both native ACs and differentiated MSCs, and their expression also changed the most during MSC differentiation and AC dedifferentiation. Further, chondrogenesis was impaired in MSCs stably overexpressing anti-miR-140-5p as demonstrated by GAG secretion, microarray and western blot analysis. SOX9 and aggrecan mRNA was unchanged, but the protein levels were greatly reduced in MSCs transduced with sh-anti-miR-140. Several possible new targets were identified and RALA, a small GTPase involved in TGF-beta/Activin signaling, was verified to be a target of miR-140-5p.
MiR-140-5p has a dramatic effect on cartilage development, and this can be explained by a positive feedback mechanism in which miR-140-5p stimulates SOX9 translation. This may occur through either a direct or an indirect mechanism.

T-3078

BIO ACCELERATES AND ENHANCES IN VITRO CARTILAGE DIFFERENTIATION OF MURINE MARROW-DERIVED MESENCHYMAL STEM CELLS

Nasrin Fallah, **Poopak Eftekhari-Yazdi**, Mohamadreza Baghaban Eslaminejad
Department of Stem Cell and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran, Tehran, Iran, Islamic Republic of

Hyaline cartilage defects exhibit a major challenge in the field of orthopedic surgery owing to its limited repair capacity. On the other hand mesenchymal stem cells (MSCs) are being regarded as potent cells with a property of cartilage regeneration. The present study was an attempt to optimize marrow-derived MSC chondrogenic culture using a small bioactive molecule referred to as BIO (6-bromoindirubin-3-oxim). MSCs from marrow of NMRI mice were extracted, culture-expanded and characterized in terms of certain surface epitopes and differentiation potential into bone , cartilage and adipose cell lineages. Micro mass culture was then established for chondrogenic differentiation (Control group). The cultures of MSC in chondrogenic medium supplemented with 0.01, 0.05, 0.1 and 1µM BIO were taken as the experimental groups. Cartilage differentiation was examined by both histological sections and real time PCR for Sox9, aggrecan and collagen II at different time points including day 5, 14 and 21 during the chondrogeneic differentiation. Moreover, to investigate whether or not Wnt pathway involved at murine MSC chondrogenic differentiation, the expression of some Wnt-specific key molecules was quantified at several time point of micro mass culture. Based on histological sections, there was seemingly more intense metachromatic matrix produced in the cultures with 0.01 µM BIO ($P<0.05$). In this experimental group, cartilage specific genes tended to be up regulated at day 14 while in the control group, the up regulation was observed at day 21. This result indicates the accelerating effect of BIO on MSC cartilage differentiation. Overall, there was statistically a significant increase ($P<0.05$) in expression level of cartilage-specific genes at cultures with 0.01µM BIO (enhancing effects) compared with those at 0.01 and 0.05 1µM BIO and the control cultures. These up-regulations appeared to be mediated through wnt pathway evident from significant up-regulation of TCF (T-cell factor) and beta-catenin molecules during the cell chondrogenic differentiation ($P<0.05$). Taken together, small molecule-BIO at 0.01µM could accelerate and enhance in vitro chondrogenesis of mouse marrow-derived MSC. This would be of great importance for cell-based treatment of cartilage defects using marrow MSCs.

T-3081

A HIGH-THROUGHPUT GENE EXPRESSION-BASED SCREEN FOR FACTORS THAT MODULATE IN-VITRO CHONDROGENESIS OF STEM CELLS IDENTIFIES OPTIMAL CONDITIONS AND NOVEL FACTORS.

Rune B. Jakobsen[1], Esben Ostrup[2], Sarah R. Herlofsen[2], Xiaolan Zhang[3], Tarjei S. Mikkelsen[4], Jan E. Brinchmann[2]

[1]Institute of basic medical sciences, University of Oslo, Oslo, Norway, [2]Norwegian Center for Stem Cell Research, Oslo University Hospital, Oslo, Norway, [3]Broad Institute of Harvard-MIT, Cambridge, MA, United States, [4]Harvard Stem Cell Institute and Broad Institute Harvard-MIT, Cambridge, MA, United States

The in vitro process of chondrogenic differentiation of mesenchymal stem cells for tissue engineering has been shown to require three-dimensional culture along with the addition of differentiating factors to the culture medium. This, however, in general leads to a phenotype lacking some of the cardinal features of native articular chondrocytes and extracellular matrix.
The factors used vary but regularly include members from the TGF beta super family and dexamethasone (DEX), sometimes in conjunction with FGF-2 and IGF-1.
However, the use of soluble factors to induce chondrogenesis has largely been studied on a single factor basis or with combinations of a few factors. We have combined a factorial design experiment with high-throughput digital mRNA profiling as a powerful tool to study in vitro chondrogenesis.
Quality control of surface markers and multipotentiality were performed on bone marrow derived MSC before embedding into alginate. A 25-factorial design of all combinations of TGF-b1, BMP-2, IGF-1, FGF-2 and DEX was then performed. Adapted designs for isoforms of TGF (-1,-2,-3) and BMP (-2, -4, -6) was also tested, giving a total of 48 different conditions. Furthermore, 38 different potential inhibitors of collagen type-I and -X synthesis were tested for their effect on chondrogenesis in a non-combinatorial design. Lysate or RNA was profiled using a custom probeset of 364 chondrogenesis related genes on the Nanostring nCounter platform.
Lysates correlated well with RNA in matched samples (mean r2 = 0.976). Graphical analysis showed temporal effects of investigated factors on wanted (14 genes among them COL2A1, COL9A1, ACAN) and unwanted (11 genes among them COL10A1, COL3A1, VCAN) gene subsets with largest separative differences after longer exposure. Main effects and interactions were therefore analysed on day 7 yielding significant positive main effects for TGFb1, BMP2 and DEX and a negative effect of FGF2 on both wanted and unwanted gene sets. Significant interactions were found for combinations of TGFb1 and BMP2 showing non-additive, non-synergistic effect on both gene sets, and for TGFb1 and DEX showing a synergistic effect on wanted genes. The combination of FGF2 with DEX or FGF with DEX and TGFb1 both had synergistic effects on the expression of unwanted genes. Examining wanted/unwanted gene ratios showed that the four highest rating conditions all contained TGFb1 and DEX in combinations with IGF1 and BMP2. We also investigated the effect of factors on early and late markers of chondrogenesis and found that TGFb1 with BMP2 simultaneously increased these markers while DEX had a positive effect on early markers such as SOX9 but an inhibitory effect on late hypertrophic markers suchs as ALPL and SPP1. This may be an indication that the traditional in vitro differentiation in part mimics secondary chondrogenesis rather than primary chondrogenesis.
Investigating novel factors as additions to the best cocktail of traditional chondrogenic factors revealed four factors intefering with cAMP-, hedgehog- or JAK-STAT-signalling to have favorable effects on COL10 expression and wanted/unwanted genes-ratios.
A cocktail of TGFb1, BMP2 and DEX is the most efficient from a gene expression perspective, but all factors beneficial to wanted genes do also increase unwanted genes. Eliminating one of these factors severely decreases expression of wanted genes, indicating that perfect differentiation to articular cartilage may not be achievable with the currently used factors.

T-3082

SILENCING DGCR8 REVEALS A CRITICAL FUNCTION FOR CANONICAL MICRORNAS ON PROLIFERATION AND DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

Stefanos Kalomoiris, Julie Beegle, Jan A. Nolta, **Fernando A. Fierro**
Stem Cell Program, University of California Davis, Sacramento, CA, United States

Multiple recent studies have evaluated the role of specific microRNAs (miRNA) in mesenchymal stem cells/multipotent bone marrow stromal cells (MSC). However, the consequence of silencing the entire miRNA machinery in MSC is poorly understood. We transduced human bone marrow-derived MSC with two different shRNAs to suppress DGCR8, an essential component for the biogenesis of all canonical miRNAs. We found that silencing DGCR8

does not affect cell viability, but strongly inhibited cell proliferation, arresting cells in the G0/G1 phase of the cell cycle. Silencing DGCR8 also strongly reduced adipogenic and osteogenic differentiation of MSC in vitro as assessed by both functional assays and expression of specific differentiation markers. To semi-quantitatively determine what miRNAs are expressed in MSC we used deep sequencing of small RNAs (RNA-seq) and found 221 miRNAs consistently expressed in MSC derived from two different donors. Surprisingly, 78% of all molecules detected belong to miR-21, miR-10 or let-7 families, suggesting critical functions for these miRNA families. Our findings demonstrate that miRNAs play a critical role during proliferation and differentiation of MSC. The fact that certain miRNA families are conserved, opens new avenues to manipulate the cells for improved differentiation and other functions.

T-3083

HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS SHOW HETEROGENOUS MRNA LEVELS OF GLUCOCORTICOID INDUCED TUMOR NECROSIS FACTOR RECEPTOR (GITR) AND ITS LIGAND (GITRL)

Cagla Z. Kopru[1], Petek Korkusuz[3], Ilgin Cagnan[4], İrem Akar Soycan[5], Gunes Esendagli[6], Duygu Uckan Cetinkaya[4], Aysen Gunel Ozcan[2]

[A]Nanotechnology and Nanomedicine, [B]Center for Stem Cell Research and Development, Department of Stem Cell Sciences, [1]Hacettepe University, Institute of Science, Ankara, Turkey, [2]Histology and Embryology, Hacettepe University Faculty of Medicine, Ankara, Turkey, [A]Center for Stem Cell Research and Development, Department of Stem Cell Sciences, [B]Center for Stem Cell Research and Development, [3]Hacettepe University, Ankara, Turkey, [4]Basic Oncology, Hacettepe University, Institute of Oncology, Ankara, Turkey

GITR (glucocorticoid induced tumor necrosis factor receptor) and its ligand GITRL are members of tumor necrosis factor (TNF) family. GITR is expressed on most of immune cell types like regulatory T-cells (Tregs). GITR/GITRL interactions were shown to play a role in immune modulation. Mesenchymal stem cells (MSC) exert an immune regulatory function by several mechanisms including suppressing T cell proliferation and recruiting Tregs. In this process GITR/GITRL interaction may represent crosstalk between the immune system by MSCs. For this purpose; this study aims to research GITR and GITRL expressions in MSCs.
In this study MSCs derived from the bone marrow (BM-MSCs) of nine healthy donors were used. BM-MSCs were expanded in vitro and characterized for their cell surface markers (CD105, CD44, CD73, CD45, CD14 and HLA-DR) by flow cytometry (FACS Aria). Also cells were successfully differentiated to adipogenic and osteogenic lineages. GITR and GITRL expressions in BM-MSCs were measured at mRNA level using real time RT-PCR (with probes from Universal Probe Library and Roche, LightCycler 480 II) and at protein level using flow cytometry. PCR results were analyzed by deltaCt method (Target geneCt - Reference geneCt) and beta-actin was used as the reference gene for normalization.
GITR and GITRL mRNA levels showed heterogeneity among donor samples. One of the donors did not show either GITR or GITRL expression. Two donors only showed low levels of GITR expression while four samples only expressed very low levels of GITRL. Additionally, two donor samples expressed both GITR and GITRL. However, flow cytometry analysis showed no GITR and GITRL protein levels.

T-3084

OPTIMIZATION OF DIFFERENTIATION MEDIA FOR DERIVATION OF NEURONS FROM HUMAN BONE MARROW MESENCHYMAL STEM CELLS

Anil Kumar[1], Sujata Mohanty[1], Anupama Kakkar[1], Balram Airan[2]

[A]Stem Cell Facility, [B]Cardiothoracic & Vascular Surgery, [1]AIIMS, New Delhi, New Delhi, India

Human bone marrow derived mesenchymal stem cells (hBM-MSCs) are a subset of self-renewable multipotent stem cells capable of differentiating into various mesenchymal lineages. Although adult stem cells are considered to be lineage-restricted, it has been demonstrated by a number of studies that hBM-MSCs can differentiate into a diverse family of cell types. Recent studies have shown that hBM-MSCs can differentiate into cells that express

properties of neural lineages both in vitro and in vivo. Using neural cells produced from autologous hBM-MSCs for transplantation in neuroreplacement therapies may eliminate the risk of immune rejection and avoid the controversial ethical issues associated with embryonic or fetal human neural stem cells.
Institute Ethics Committee (IEC) & Institutional Committee for Stem Cell Research and Therapy (IC-SCRT) ethical clearance was obtained before initiation of the study. cryopreserved hBM-MSCs of patients (n=3) of second passage (2P) were revived and expanded in LG-DMEM supplemented with 10% FBS at 37ºC/5%CO2. The culture was established and cells were characterized on the basis of their morphology, plastic adherence, and surface marker profile. hBM-MSCs were characterized for the expression of vimentin and fibroblast specific protein (FSP) by immuno fluorescence (IF) staining; surface markers CD105, CD90, CD29, CD73, HLA Class I & Class II, CD 34/45 by flowcytometry. The hBM-MSCs after characterization at third passage (3P) were induced into neuronal lineage using two different protocols. Neurobasal media, B-27 supplement, L-Glutamine and PenStrep were common to both the protocols. Apart, from this, Protocol I consisted of epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2), while Protocol II consisted of sonic hedge-hog (Shh), FGF-2, and FGF-8 as inducers. Induction was terminated at day 12 and cells were proceeded for characterization for neuronal lineage specific markers βIII-tubulin, microtubule associated protein 2 (MAP2), neurofilament (NF) and tyrosine hydroxylase (TH).
hBM-MSCs cells were found to be positive for vimentin, CD105, CD90, CD29, CD73, HLA I and negative for FSP, HLAII and CD34/45. Differentiation studies revealed expression of MAP2 and βIII-tubulin by IF and flowcytometry and NF and TH by quantitative RT-PCR (qRT-PCR) in both the treatment groups. The fluorescence intensity for the expression of MAP2 and βIII-tubulin was found to be higher in cells induced by Protocol I as compared to Protocol II by IF. The efficiency of differentiation based on the expression of MAP2 and βIII-tubulin by flowcytometry was higher in Protocol I as compared to Protocol II. The median values of the MAP2 and βIII-tubulin expression was 34 and 39 respectively as compared to median values of 21 and 20 respectively for the cells induced by Protocol II. The expression of TH and NF was higher in cells induced by Protocol I as compared to Protocol II as revealed by qRT-PCR. The fold increase in the expression of TH and NF was 2.83±1.50 and 3.49±0.42 fold for the cells induced by Protocol I as compared to cells induced by Protocol II for which the fold expression of TH and NF was 1.60±0.03 and 2.11±0.99 fold respectively. The data represent mean±SD of three samples.
This preliminary study reveals that Protocol I is feasible and cost effective method to transdifferentiate hBM-MSC efficiently into neurons using minimal growth factors and single inducer.

T-3085

Hyperbaric Oxygen Therapy Promotes Neosculogenesis In Streptozocin-Induced Diabetes Mellitus Type 1 Mice In Vivo via Wnt Signaling Pathway

Tatyana N. Milovanova[1], Gunalp Uzun[2], Elena M. Sorokina[2], Jonni S. Moore[3], Steven R. Thom[2]
[1]Univ of Pennsylvania, Philadelphia, PA, [A]Medicine, [B]Pathology and Flow Cytometry, [2]Univ of Pennsylvania, Philadelphia, PA, United States

Our recent research showed that hyperbaric oxygen therapy could promote the mesenchimal stem/progenitor cell (SPCs) proliferation and differentiation in diabetic patients wound healing process via reactive oxygen /reactive nitrogen species (ROS/RNS)-dependent mechanism. Adipose-derived mesenchymal stem cells (ASCs) through the VEGF secretion induce migration and proliferation of endothelial cells, increasing the vascularity of wound bed. We hypothesized that oxidative stress from hyperbaric oxygen (HBO_2, 2.8 ATA for 90 min daily) exerts a trophic effect on ASCs in streptozocin-induced diabetes mellitus type 1 mouse model via ROS/RNS and activation of Wnt -signaling pathway mechanism. Adipose-derived stem/progenitor cells (Sca-1+/CD31-/DAPI-), were sorted from omentum of additional WT mouse, labeled with the fluorescent dye 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE) and Matrigel plugs were injected subcutaneously into both sides of the thoracic vertebrae before the HBO_2 treatment. The activation Wnt-signaling pathway was detected by Dickkopf-1 (DKK-1) Wnt antagonist. In combination, HBO_2 and ASCs *in vivo* demonstrated cumulative effect. Vascular channels formation identified by $CD34^+$ SPCs staining was significantly reduced in diabetic animals. In the Matrigel with seeded ASCs and Dkk1 accelerated channel development after HBO_2 treatment along with cell differentiation based on the expression of neovasculogenic sur-

face markers and cell cycle entry was identified. $CD34^+$ SPCs of diabetic animals in blood and bone marrow defined down regulated thioredoxin-1 (Trx1), Trx reductase, hypoxia-inducible factors (HIF)-1, -2, and -3, phosphorylated mitogen-activated protein kinases, vascular endothelial growth factor, and stromal cell-derived factor-1 in. Cell recruitment to Matrigel and protein synthesis responses was abrogated in STZ-mice improved by adding Dkk-1 to the Matrigel. Thioredoxin system activation leads to elevations in HIF-1 and -2, followed by synthesis of HIF-dependent growth factors. Conclusion: Dkk-1 enchances angiogenic properties of ASCs, HBO_2activates a physiological redox-active autocrine loop in recruited SPCs and increased paracrine secretion in seeded ASCs, via Wnt-dependent mechanism, resulted in the stimulated neovasculogenesis.

T-3086

HUMAN ADIPOSE DERIVE MESENCHYMAL STEM CELLS ATTENUATE LIVER ISCHEMIA-REPERFUSION INJURY AND PROMOTE LIVER REGENERATION

REZA SAIDI[1], B. RAJESHJUMAR[1], O. WALTER[2], A. SHARIFTABRIZI[3], K. DRESSER[4]
[A]SURGERY-TRANSPLANTATION, [B]PATHOLOHY, [1]University of Massachusetts Medical School, WORCESTER, MA, United States, [D]PATHOLOGY, [1]University of Massachusetts Medical School, WORCESTER, MA, United States

HUMAN ADIPOSE DERIVE MESENCHYMAL STEM CELLS ATTENUATE LIVER ISCHEMIA-REPERFUSION INJURY AND PROMOTE LIVER REGENERATION

Liver ischemia-reperfusion injury (IRI) is a well-known cause of morbidity and mortality following Liver transplantation. Effective treatment strategies aimed at reducing hepatic IRI injury and accelerating liver regeneration could offer major benefits in LT.
Recent reports have demonstrated the capacity of mesenchymal stem cells (MSCs) to specifically be involved in the repair of organ tissue. We investigated the effect of human adipose derive MSC (HAD-MSC) on IRI and liver regeneration.
HAD-MSC were isolated from the perinephric fat of living kidney donors. Humanized mice (NOD-*scid ILr2*^{null}) were subjected to 30 minutes of 70% partial IRI with and without 70% partial hepatecotomy (PH). Animals were treated with intravenous HAD-MSC (1-2x 10^6) or normal saline. IRI injury was evaluated using serum levels of alanine aminotransferase (ALT), serum interleukin-6 (IL-6), IL-1, TNF-alpha and specific markers of regeneration (BrdU staining and PCNA) and hematoxylin and eosin staining.
Histology, serum IL-6, IL-1, TNF-alpha and ALT release revealed that HAD-MSC provided significant protection against IRI+-PH compared with controls Improved animal survival and increased number of BrdU positive cells were observed in treated animal which underwent IRI/PH compared to control group.
These data indicate that HAD-MSC plays a key role in IRI and liver regeneration. HAD-MSC represents a potential strategy to reduce IRI and promote regeneration in liver transplantation.

T-3088

MIR21 GOVERNS HUMAN MESENCHYMAL STEM CELL PROPERTIES

Ourania Trohatou[1], Dimitra Zagoura[1], Vasiliki Bitsika[1], Kalliopi I. Pappa[2], Nicholas P. Anagnou[1], Maria G. Roubelakis[1]
[A]and BRFAA, Cell and Gene Therapy Laboratory, [1]University of Athens, Medical School, Athens, Greece

MicroRNAs (miRNAs) are small, noncoding RNA molecules of 20-23 nucleotides that regulate gene expression by interacting with specific sites in the 3' untranslated regions (3'UTRs) of their target mRNAs. miRNAs have recently been shown to act as regulatory signals for maintaining stemness and determining the fate of adult and fetal stem cells, such as mesenchymal stem cells (hMSCs). hMSCs constitute a population of multipotent stem cells that can be easily expanded in culture and are able to differentiate into many lineages. Our group has isolated two subpop-

ulations of fetal MSCs from amniotic fluid (AF) known as spindle (SS) and round-shaped (RS) cells and characterized them based on their phenotypes, pluripotency, proliferation rates and differentiation potentials. In this study, we analyzed the miRNA profile of MSCs derived from AF, bone marrow (BM) and umbilical cord blood (UCB). We initially identified 67 different miRNAs that were expressed in all three types of MSCs but at different levels, depending on the source. More specifically, 32 miRNAs were differentially expressed between the SS-AF-MSCs and RS-AF-MSCs. A more detailed analysis revealed that miR-21 was expressed at higher levels in RS-AF-MSCs and BM-MSCs compared with SS-AF-MSCs. The transcription factor Sox2 was identified as a target gene containing predicted miR-21 binding sites. We further demonstrated for the first time a direct interaction between miR-21 and the pluripotency marker Sox2. The induction of miR-21 strongly inhibited Sox2 expression in SS-AF-MSCs, resulting in reduced clonogenic and proliferative potential. Statistically significant decreases in Oct4 and Nanog were also observed at both the mRNA and protein levels in SS-AF-MSCs expressing miR-21 compared to control cells. In addition, miR-21 induction in SS-AF-MSCs led to a significant suppression of Cyclin D1, Cyclin E1 and Cyclin A, resulting in cell cycle arrest. Strikingly, the opposite effect was observed upon miR-21 inhibition in RS-AF-MSCs and BM-MSCs, which led to an enhanced proliferation rate. However, no alterations in RS-AF-MSC and BM-MSC morphology were observed upon miR-21 inhibition. Finally, miR-21 induction accelerated osteogenesis and impaired adipogenesis and chondrogenesis in SS-AF-MSCs. Our data suggest that miR-21 may act as a regulator of the clonogenic potential, proliferation rate and differentiation properties of AF-MSCs, most likely via the direct suppression of the transcription factor Sox2.

T-3091

IODINE PROMOTION OF OSTEOGENIC DIFFERENTIATION ENHANCES STEM CELL-BIOSCAFFOLD BASED SUBCHONDRAL BONE REGENERATION

Yangzi Jiang[1], Longkun Chen[3], Shufang Zhang[4], Tong Tong[5], Wei Zhang[6], Wanlu Liu[7], Rocky S. Tuan[2], Yin Xiao**[8], Hongwei Ouyang*[9]

[A]Department of Orthopaedic Surgery, [B]Department of Orthopaedic Surgery,Center for Cellular and Molecular Engineering, [1]University of Pittsburgh, Pittsburgh, PA, United States, [2]Zhejiang Xingyue Biotechnology Co.Ltd., Hangzhou, China, [A]Center for Stem Cell and Tissue Engineering;Zhejiang Provincial Key Laboratory of Tissue Engineering, [B]School of Medicine, [3]Zhejiang University, Hangzhou, China, [4]Zhejiang Provincial Key Laboratory of Tissue Engineering and Regenerative Medicine, Hangzhou, China, [5]School of medicine, Zhejiang Univeristy, Hangzhou, China, [6]Institute of Health and Biomedical innovation, Queensland University of Technology, Brisbane, Australia, [7]Zhejiang Provincial Key Laboratory of Tissue Engineering and Regenerative Medicine, School of Medicne,Zhejiang University, Hangzhou, China

BACKGROUND: Povidone-iodine [polyvinylpyrrolidone-iodine complex (PVP-I)] is widely utilized as an antiseptic agent for lavation during joint surgery; however, the biological effects of PVP-I on joint tissue cells are not clear In this study, we examined the biocompatibility and biological effects of PVP-I on joint tissue cells towards optimization of cell-scaffold based joint repair.

METHODS: Joint tissue cells, including human cartilage derived progenitor cells (CPCs) and human bone marrow derived mesenchymal stem cells (BMSCs) were isolated. The concentration-dependent effects of PVP-I on cell proliferation, migration and differentiation were evaluated. The efficacy of a PVP-I loaded bi-layer collagen scaffold for osteochondral defect repair in a rabbit model was investigated .

RESULTS: Micromolar concentrations of PVP-I was found to not affect cell proliferation, nor CPC migration and ECM production. Interestingly, micromolar PVP-I promoted osteogenic differentiation of BMSCs, evidenced by upregulated expression of RUNX2 and Osteocalcin, as well as increased mineralization by cells seeded on 3-D scaffold. PVP-I treatment of collagen scaffold significantly increased fibronectin binding onto the scaffold surface, and collagen type I protein synthesis of seeded BMSCs. Implantation of PVP-I treated collagen scaffolds into a rabbit osteochondral defect significantly enhanced subchondral bone regeneration at 6 weeks post-surgery compared to the scaffold alone (subchondral bone histological score of 8.80±1.64 *vs* 3.8±2.19; $p<0.05$).

CONCLUSIONS: The biocompatibility and pro-osteogenic activity of PVP-I on joint tissue cells and the enhanced subchondral bone formation in PVP-I treated scaffold in vivo suggest potential utility of PVP-I for osteochondral repair.
(Support: Commonwealth of Pennsylvania Dept of Heath)

T-3092

THE HETEROGENEOUS NATURE OF MESENCHYMAL STROMAL CELLS (MSCS) IS PARTIALLY DUE TO INHERENT DIFFERENCES IN GENE EXPRESSION

Meirav Pevsner-Fischer, Sarit Levin, Sivan Kagan, Hila Lifshitz, Ada Weinstock, Diana Gataulin, Dov Zipori
Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel

Objective: to study the molecular mechanism underlying heterogeneity in MSCs
The heterogeneity of bone marrow (BM)-mesenchymal stromal cells (MSCs) is a well-known phenomenon. Individual MSC preparations differ in morphology, growth rate and differentiation potentials. However, the molecular mechanism underlying this phenomenon is unknown. We examined a series of independently derived MSC preparations for their response to activation by several toll-like receptor (TLR) ligands. We found that the differentiation of independent populations of MSCs were highly divergent in response to TLR activation. Single cell clones from an individual MSC responded differently and often in opposing manner to the same TLR ligands. Gene chip analysis revealed that independently derived MSCs differ in expression patterns of many cellular pathways, including the TLR pathway. Molecular and functional analysis confirmed that TLR related gene expression varies among MSCs. For example, we found a correlation between IL-6 secretion by MSCs upon addition of LPS and LPS binding protein (LBP) expression, i.e., MSCs that did not express LBP, also did not secrete IL-6 in response to LPS. Addition of recombinant LBP rescued the response to LPS. Differential genes expression was recorded not only of TLR pathway, but also in many other pathways. This trait is general to MSCs in many if not all signaling pathways such as WNT, differentiation and cell cycle related genes. We speculate that inherent differential gene expression partially accounts for the existence of functionally different MSC clones in the bone marrow. This allows fine-tuning of the response to stress signals and regeneration of the tissue, while preventing progenitor depletion.
The first two authors contributed equally to the presented work.
Table of Contents

T-3093

PLATELET-DERIVED FACTORS PRESERVE STEMNESS OF MESENCHYMAL CELLS IN VITRO AND MAINTAIN THEIR ENDOCHONDRAL BONE AND MARROW NICHE FORMATION POTENTIAL IN VIVO VIA KINASE- AND GPCR-DEPENDENT PDGF RECEPTOR SIGNALLING

Dirk Strunk[1], Nathalie Etchart[3], Andreas Reinisch[4], Anna Ortner[1], Nicole A. Hofmann[1], Katharina Schallmoser[2]
[A]Experimental and Clinical Cell Therapy Institute, [B]Transfusion Medicine Department, [1]Paracelsus Medical University, Salzburg, Austria, [2]Stem Cell Research, Medical University, Graz, Austria, [3]ISCBRM, Stanford University, Stanford, CA, United States

Most non-hematopoietic stem cells require propagation in optimized media including variable preparations of fetal bovine serum as an essential growth supplement to obtain the sufficient quality and quantity of cells for research and therapy. We and others have previously shown that human platelet lysate (HPL) can replace FBS for human mesenchymal stem/progenitor cell (MSC) propagation in vitro even boosting MSC proliferation. Preliminary evidence indicates increase of osteogenic differentiation by HPL in vitro. The underlying mechanism is not known.
To study the mode of action of the natural platelet-derived growth factors on therapeutic MSC behaviour we generated 11 pairs of human BM-derived MSCs that were separated 50:50 immediately after harvest before any serum contact and cultured exclusively either in the absence of xenogenic proteins, using HPL (http://www.jove.com/video/1523) as alternative cell culture supplement (HPL-MSC), or in standard culture medium with selected lot of FBS (FBS-MSC). The influence of culture conditions was tested in vitro by evaluating differentiation capacity and

phenotypic changes and in vivo using a subcutaneous ectopic human bone and marrow niche (HuNiche; Blood 119:4971) formation model in immune-compromised NSG mice (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) including re-transplantation.
Both cell types were phenotypically virtually indistinguishable regarding MSC criteria and differentiated osteo-, adipo- and chondrogenically in standard assays in vitro. Interestingly, cartilage fragments created in a 3D assay in vitro from HPL-MSC were on average 4 times heavier than those generated with FBS-MSC. In the HuNiche model in vivo HPL-MSC from all 11 donors (100%) spontaneously initiated bone formation by an endochondral mechanism in NSG mice. Implants from 7/11 donors attracted complete mouse bone marrow indicating that HPL-MSC retain the capacity to establish a bone marrow-supporting niche. Only 2/11 corresponding FBS-MSC lines formed bone and just 1/11 established a marrow niche.
Extensive phenotypic analysis revealed that the stage-specific embryonic antigen SSEA-4 was higher expressed on HPL-MSC vs. FBS-MSC, suggesting that stemness or multipotentiality could be maintained by platelet-derived factors. Accordingly, higher SSEA-4 expression correlated with the capacity of HPL-MSC to create a hematopoietic bone marrow niche in vivo. In a downstream target screen, cholera toxin but not pertussis toxin inhibited osteogenesis in vitro and in vivo implicating a role of GPCR-alpha-S. Transferring HPL-MSC for at least one passage in FBS medium or Imatinib treatment to inhibit PDGF-R-beta kinase (which can co-signal with GPCR-alpha) equally resulted in a loss of SSEA-4 surface expression paralleled by a loss of in vivo differentiation potential. Specific PDGF-R phosphorylation was found in HPL- but not FBS-MSC despite equal surface expression of PDGF-R-beta in both cohorts. Re-isolation of human MSC and serial re-transplantation was only successful with HPL-MSC forming bone in secondary recipients suggesting longer term maintenance of MSC stemness in vivo.
We conclude that platelet-derived factors and PDGF-R signalling are crucial for maintaining MSC multipotentiality mediated by HPL.

T-3094

A NEW CELL INTERACTION MECHANISM FOLLOWING HUMAN MESENCHYMAL STEM CELL PRE-CONDITIONING

Daniel J. Kota, Bryan DiCarlo, Robert A. Hetz, Scott D. Olson
Pediatric Surgery, University of Texas Health Science Ctr-Houston, Houston, TX, United States

The ease of culturing Mesenchymal Stem cells (MSCs), combined with its stemness and safety have led investigators to explore the potential of MSC as a therapeutic approach for the replacement of injured/dead cells in many disease models. However, advances in the MSC field have challenged the replacement therapy paradigm and indicated that MSCs could improve disease outcome without the need for engraftment and differentiation. In this new paradigm, MSC's true 'plasticity' may in fact lie on its capacity to differently respond to a wide array of activation signals, often leading to heterogeneous and sometimes antagonistic therapeutic properties. Here, we showed that MSC activated with the TLR3 activator Poly I:C, but not LPS or TNF-α, are activated to express a highly complex Hyaluronic acid (HA) structure, which significantly increases the binding of U937 monocytic leukemia cell line, while maintaining its immunossupressive properties. Given the importance of HA interactions in many biological activities, including cell adhesion, inflammation and cancer, HA -expressing MSCs may represent a new potential therapeutic approach.

T-3095

MIGRATION, PROLIFERATION AND DIFFERENTIATION OF CORD BLOOD MESENCHYMAL STROMAL CELLS TREATED WITH HISTONE DEACETYLASE INHIBITOR VALPROIC ACID

Leah A. Marquez-Curtis[1], Yuanyuan Qiu[2], Anna Janowska-Wieczorek[3]
[A]R&D, [B]R & D, [1]Canadian Blood Services, Edmonton, AB, Canada, [2]Univ of Alberta, Edmonton, AB, Canada

Mesenchymal stromal cells (MSC) have great potential for cellular therapies as they can be directed to differentiate into certain lineages or to exert paracrine effects at sites of injury. The interactions between the chemokine stromal cell-derived factor (SDF)-1 (also known as CXCL12) and its receptors (CXCR4 and CXCR7) play pivotal roles in migra-

tion of cells to the injury site where the local concentration of SDF-1 increases. However, the low surface expression of CXCR4 on cultured MSC limits their ability to migrate towards SDF-1. We previously demonstrated that valproic acid (VPA), an inhibitor of histone deacetylase, increases the CXCR4 expression and chemotaxis of cord blood (CB) hematopoietic stem/progenitor cells. As the ability of MSC to migrate towards injury sites is important for their clinical application, in this study we evaluated whether VPA increases the migration of CB-derived MSC towards SDF-1, and has an effect on proliferation and differentiation of CB MSC. CB-derived MSC were characterized according to the ISCT minimal criteria (phenotype: positive for CD105, CD73, and CD90, negative for CD34 and CD45, in vitro differentiation to osteoblasts and chondroblasts). MSC at passage 4 to 6 were exposed to various concentrations of VPA (1 mM, 5 mM, 10 mM) and times (3 h or 6 h) and were evaluated for: i) expression of CXCR4 and CXCR7 using quantitative RT- PCR and flow cytometry; ii) invasion/migration across the reconstituted basement membrane Matrigel towards SDF-1; iii) expression of matrix metalloproteinases (MMPs), especially those known to regulate the migration of MSC, namely membrane type 1 (MT1)-MMP and MMP-2, using qRT-PCR and zymography; iv) proliferation using LUMENESC-96 assay (HemoGenix Inc.); v) expression of pluripotency genes SOX-2 and Oct-4; and vi) differentiation to osteocytes and chondrocytes and expression of myogenic markers myogenin and MyoD. We found that short-term (3 h and 6 h) treatment with VPA increased the gene expression of CXCR4 and CXCR7 in a dose-dependent manner but not surface expression. VPA treatment primed the trans-Matrigel migration of MSC towards a low gradient (20 ng/ml) of SDF-1 by nearly three-fold, and this priming effect was inhibited by the CXCR4 antagonist AMD3100 ($p<0.001$) and the CXCR7 antagonist CCX733 (ChemoCentryx Inc.; $p<0.05$). Moreover, treatment of MSC with 5 mM VPA for 3 h increased the gene expression of MMP-2 (1.5-fold) and secretion of pro-MMP-2 (1.4-fold) in MSC. MSC proliferation increased after a 3 h exposure to lower concentrations of VPA (1 mM and 5 mM), and gene expression of pluripotency markers SOX-2 and Oct-4 increased up to 9-fold and 4-fold, respectively. The short-term exposure of MSC to VPA had no effect on their differentiation to osteocytes and chondrocytes but increased the gene expression of myogenin and MyoD up to 4-fold and 2.5-fold, respectively. In conclusion, our study indicates that VPA enhances the migration of CB MSC towards SDF-1 by increasing the expression of CXCR4 and CXCR7, as well as the levels of MMP-2 suggesting that VPA may be used for ex vivo treatment of MSC to increase their recruitment to sites of injury without compromising their ability to differentiate into osteocytes, chondrocytes and myocytes.

T-3096

AGE-RELATED CHANGES TO SERUM-RESPONSE FACTOR LEVELS IN HUMAN MESENCHYMAL STEM CELLS ARE INVOLVED IN IN VITRO SKELETAL MUSCLE DIFFERENTIATION CAPACITY AND IN VIVO ENGRAFTMENT

Chiao-Hsuan Ting, Pai-Jiun Ho, Men-Luh Yen, B. Linju Yen
Natl Health Res Institutes, Zhunan Taiwan, Taipei City, Taiwan

Purpose(s): Skeletal muscle (SkM) comprise approximately 40% of human body weight. This important tissue undergoes progressive decline with aging, which is poorly reversed by its endogenous stem cell population, the satellite cell. Therefore, we asked whether human sources of developmentally early-stage mesenchymal stromal cells (hDE-MSCs) could be a cellular source for repair of SkM.
Procedures: In vitro muscle differentiation of multilineage hDE-MSCs from three sources was performed using standard protocols, with SkM differentiation ascertained by gene/protein expression and functional assay analysis. A mouse model of SkM injury was used to assess for in vivo engraftment of injected hDE-MSCs.
Findings: hDE-MSCs differentiated most efficiently towards a SkM lineage, with only SkM-differentiated hDE-MSCs but not adult bone marrow (BM) MSCs incorporating into in vivo host injured SkM. Compared to adult BMMSCs, hDE-MSCs expressed higher levels of serum response factor (SRF), a transcription factor important for SkM-lineage commitment, and knockdown of SRF in hDE-MSCs abrogated both in vitro and in vivo SkM differentiation capacity.
Controls: Adult BMMSCs, the most commonly used source of multilineage MSCs, were used as control cells.
Conclusion: Our data demonstrate the important role of SRF in SkM differentiation, and hDE-MSCs as possible sources for cell therapy of SkM-related pathologies.

T-3097

BENEFICIAL EFFECT OF SYSTEMIC DELIVERY OF HUMAN MESENCHYMAL STROMAL CELLS COMBINED WITH GROWTH FACTORS IN DYSTROPHIC MICE.

Mariane Secco, Eder Zucconi, Carlos Bueno Jr., Natassia M. Vieira, Camila Almeida, Mayra Pelatti, Paolo Bartolini, Mariz Vainzof, Elen H. Miyabara, Oswaldo K. Okamoto, Mayana Zatz
University of São Paulo, Sao Paulo, Brazil

ABSTRACT
Progressive muscular dystrophies are a group of diseases characterized by degeneration of skeletal muscle. Strategies for the development of a muscular dystrophy therapy have focused on the possibility of restoring the defective muscle protein through stem cell engraftment or on delivery of growth factors to ameliorate muscular pathology symptoms. Here we investigated whether the combination of both strategies could also be a useful approach to treat this group of diseases. In order to address this question, we analyzed the outcomes of the association of both IGF-1 - a growth factor known to improve muscle regeneration - and human mesenchymal stromal cells from umbilical cord tissue (MSCs) on muscle functional performance of the murine model for congenital muscular dystrophy type 1A - B6.WK-LAMA2dy/2j. Dystrophic mice were assigned into four groups: untreated dystrophic group, IGF-1 treated group (2 mg/Kg/day for 8 weeks, via osmotic pumps), MSCs injected group (1x106 cells systemically injected, once a week for 2 months), and IGF-1+MSCs treated group. We demonstrated that MSCs were able to reach the skeletal muscle of LAMA2dy/2j dystrophic mice, through systemic delivery, without immunosuppression. The best results were observed in the last group, showing, for the first time, that IGF-1 injected systemically together with MSCs significantly improved muscle strength in dystrophic mice. No human muscular protein in skeletal muscle of MSCs injected mice was observed suggesting that it is unlikely that MSCs have differentiated into muscle cells. However, mice from IGF-1+MSCs treated group presented an evident reduced muscle inflammation and fibrosis. Supporting these findings, several evidences have indicated that terminal differentiation is not a major determinant for the success of stem cell therapy and that MSCs may contribute to tissue repair or protection through the production of trophic factors, including growth factors, cytokines and antioxidants, some of which providing the basis for their capacity to modulate inflammatory and/or immune responses. Our results suggest that a combined treatment with IGF-1 and MSCs are clinically beneficial and, therefore, should be considered as a potential therapeutic approach in muscular dystrophies. Further studies are underway in order to investigate the effects of soluble factors released by MSCs in dystrophic muscle function.

T-3098

N-CADHERIN IS A USEFUL PROSPECTIVE CELL SURFACE MARKERS FOR HUMAN MESENCHYMAL STEM CELLS THAT DIFFERENTIATE INTO CARDIOMYOCYTES.

Hisako Ishimine[1], Norio Yamakawa[1], Mari Sasao[2], Mika Tadokoro[2], Daisuke Kami[3], Makoto Tokuhara[4], Hajime Ohgushi[2], Akihiro Umezawa[3], Makoto Asashima[1], Akira Kurisaki[1]
[1]Research Center for Stem Cell Engineering, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan, [2]Health Research Institute, National Institute of Advanced Industrial Science and Technology, Amagasaki, Japan, [3]Department of Reproductive Biology and Pathology, National Research Institute for Child Health and Development, Setagaya, Japan, [4]Department of Surgery, National Center for Global Health and Medicine, Shinjyuku, Japan

Myocardial infarction caused by chronic atherosclerosis of the coronary artery could induce heart failure with high mortality. Because myocardium has very limited ability of regeneration, heart transplantation is currently considered to be the most effective treatment for severe heart failure. However, the significant shortage of donor hearts and chronic graft versus host disease after allogeneic heart transplantation hinders wide application of this method. Alternatively, stem cell therapy is expected for the regenerative medicine of this disease. Mesenchymal stem cells (MSCs) derived from adipose tissue and bone marrow differentiate into multiple mesoderm-type cells, such as osteoblasts, chondrocytes, adipocytes. Under certain in vitro culture conditions, MSCs can also differentiate

into neuron, skeletal muscle and cardiomyocytes. Particularly, they do not differentiate into the cardiomyocytes at high efficiency. To improve the differentiation efficiency of MSCs into cardiomyocytes, we explored prospective cell surface markers that enable to enrich cardiomyogenic progenitors from MSCs derived from human bone marrow (BMSCs) and adipose tissues (ASCs). We analyzed the cell-surface expressions of trans-membrane protein markers that are essential during heart development using various human BMSCs, ASCs and various human MSC cell lines. When these cells were differentiated into beating cardiomyocytes using a mouse primary cardiomyocytes coculture system, a good correlation was observed between cell-surface expression of N-cadherin and the differentiation efficiency of MSCs into cardiomyocytes. Moreover, DNA microarray analysis revealed that N-cadherin-positive cells isolated with magnetic cell sorting showed significantly higher expression of several cardiomyogenic progenitor cell-specific transcription factors such as *Nkx2.5, Hand1,* and *Gata4*. Although the overall absolute expression level of cardiomyocyte-specific terminal markers was low, expression levels of the cardiomyocyte-specific markers were increased in the N-cadherin-positive fractions. Finally, we confirmed that N-cadherin-positive MSCs purified by MACS showed apparently higher ability to differentiate into cardiomyocytes than the N-cadherin-negative population. Our results suggested that N-cadherin could be a prospective cell surface marker for MSCs that have higher cardiomyogenic potential.

T-3101

NEURONAL DIFFERENTIATION OF MESENCHYMAL STEM CELLS WITH FORCED EXPRESSION OF PROGRANULIN

Kit M. Tsang[1], **Kam S. Tsang**[1], Gang Lu[2], Ho K. Ng[1]

[A]Department of Anatomical and Cellular Pathology, [B]Department of Surgery, [1]The Chinese University of Hong Kong, Shatin, Hong Kong

Mesenchymal stem cells (MSC) encompass the capacity of differentiation into multiple lineages. In addition, their roles in the paracrine signaling of a spectrum of growth factors and cytokines suggested the cell-based therapeutic potential of MSC against a variety of diseases attributed to cell loss and degeneration. *Progranulin* (*PGRN*) was reported to involve in various physiologic processes including early embryogenesis. The putative effects of *PGRN* on neuronal induction of MSCs had not been well defined. In this study, we transduced *PGRN* into MSC derived from normal bone marrow (n=3) by using adenovirus transfection and compared the efficacy of neuronal induction of *PGRN*-transduced and non-transduced MSC. *PGRN*-transduced MSC displayed a di-polar, spindle-shaped, fibroblast-like morphology as the non-transduced counterparts, however a slower growth kinetics was noted (population doubling time in hours: *PGRN*-transduced MSC vs. non-transduced MSC; 32.8 vs. 30.2, $p<0.005$). Quantitative polymerase chain reaction demonstrated that forced expression of *PGRN* in MSC down-regulated mesodermal genes, *Brachyury* and *Gata-4*, compared to those of non-transduced MSC. There was no change of the gene expressions of the neural lineage, including *Sox-2, Sox-1, Musashi-1, Pax-6 and Nestin* in both *PGRN*-transduced and non-transduced MSC. Conversely, *PGRN*-transduced MSC exhibited a 1.5-folded increase of the gene expression of *ciliary neurotrophic factor (CNTF)* among the panel of growth factors of *brain-derived neurotropic factor* (*BDNF*), *fibroblast growth factor* (*FGF*), *glial cell line-derived neurotrophic factor*, *nerve growth factor*, *tumor necrosis factor* and their receptors. Upon completion of neuronal induction with SHH, FGF-8, basic FGF and BDNF for 12 days, the mean number of cells derived from *PGRN*-transduced cell cultures was comparable to that of non-transduced cultures, and similar proportions of cells with neurite bifurcation were also noted (*PGRN*-transduced cells vs. non-transduced cells; 11.2% vs. 10.7%, $p=0.88$). Immunofluorescence staining of neuronal markers, β-tubulin III and tyrosine hydroxylase, revealed no significant difference between the numbers of positive cells derived from *PGRN*-transduced and non-transduced MSC in induction cultures, however protruding processes of derived cells from *PGRN*-transduced MSC were noted to be longer than those of non-transduced MSC (mean length in μm: 173.3 ± 36.8 vs. 149.7 ± 48.2, $p<0.01$). An up-regulation of gene expressions of *Pax-6*, *Musashi-1* and *CNTF* in neuronal differentiation cultures of *PGRN*-transduced MSC was evident implying that forced expression of *PGRN* enhanced the neuro-ectodermal fate of MSC and paracrine signaling. Data of the study suggest that *PGRN*-transduced MSC might play a role in the neuronal differentiation of MSC applicable to the cell therapy for neurologic diseases related to cell loss and degeneration.

T-3102

MICRORNA-ENHANCED NEUROSPHERE FORMATION FROM MESENCHYMAL STEM CELLS

Jing Ye ZHOU, Pi Chu LIU, Meng LEI, Yao LIU, Kam Sze Kent TSANG, Gang* LU, Wai Sang POON
Division of Neurosurgery, Department of Surgery, The Chinese University of Hong Kong, Shatin, Hong Kong

MicroRNAs play an essential role in regulating MSC differentiation. To better understand whether miroRNAs are involved in the neurosphere formation from MSC, we examined miRNA expression profiles of MSC-induced neurosphere and MSC using microarray technique. Total 22 different miRNAs were found with different expression between MSC and MSC-Neurosphere. Among which we identify 9 as brain-enriched miRNAs. To further identify the function of above brain-enriched miRNAs, these miRNAs were conducted into MSC separately by lentivirus vectors to investigate those functions on neurosphere formation. We found that two brain-enriched MicroRNAs, MiR-149 and -370 have significant role in regulation of MSC neurosphere formation. Overexpression of miR-149 or -370 in MSC can enhance the formation of neurosphere. Differential gene expression analysis, in combination with computational searches, defined 12 putative target genes for miR-149 and -370 in hMSCs. Western blot and 3- untranslated region reporter assays confirmed the repressor elements 1-silencing factor (REST) as a direct target of miR-149 and -370 in hMSCs. REST is critical transcription coordinate repressor in regulating neuronal differentiation and also is found high expression in proliferating mesenchymal stem cells but is rapidly downregulated as the transcription of neuronal-specific genes are actived . We showed that exogenously expressed REST could partially rescues the phenotypes associated miR-149 and -370 overexpression. This study demonstrates a novel miRNA-mediated mechanism in MSC differentiation and suggests that miR-149 and -370 are important mediator s of neurosphere formation. This work was supported by CUHK Direct Grant (2041778).

T-3103

ROLE OF NOTCH SIGNALING IN THE MAINTENANCE OF HUMAN MESENCHYMAL STEM CELLS UNDER HYPOXIC CONDITIONS.

Hiroyuki Moriyama[1], Mariko Moriyama[1], Akifumi Matsuyama[2], Takao Hayakawa[1]
[1]Kinki Univ, Higasi-Osaka, Japan, [2]Department of Somatic Stem Cell Therapy and Health Policy, Foundation for Biomedical Research and Innovation, Kobe, Japan

Human adipose-derived mesenchymal progenitor cells (hADMPCs) are an attractive material for cell therapy and tissue engineering because of their multipotency and the ease of availability without serial ethical issues. However, their limited lifespan in in vitro culture system hinders the therapeutic applications of hADMPCs. Some somatic stem cells including hADMPCs are known to be localized in hypoxic region, thus hypoxia may be beneficial to ex vivo culture of these stem cells. They exhibit a high level of glycolytic metabolism despite the presence of high oxygen, and further increase their glycolysis rate under hypoxia. However, physiological role of glycolytic activation and its regulatory mechanisms is still incompletely understood.
Unlike somatic stem cells, embryonic stem cells and induced pluripotent stem cells are immortal under standard culture conditions. Interestingly, these cells greatly rely on glycolysis for energy production even under high oxygen conditions. This phenomenon has been known as the Warburg effect, originally described for cancer cells. It has also been demonstrated that enhanced glycolysis is involved in cellular immortalization through reduction of intrinsic ROS production. Since accumulation of intrinsic ROS levels could be one of the major reasons for replicative senescence, enhancing glycolysis in cultured cells might improve the quality of the cells by suppressing premature senescence.
Here we show that Notch signaling is required for glycolysis regulation under hypoxic condition. Our results demonstrate that 5% O_2 dramatically increased the glycolysis rate, improved the proliferation efficiency, prevented the senescence, and maintained the multipotency of hADMPCs. Intriguingly, these effects were not mediated by hypoxia-inducible factor (HIFs), but Notch signaling pathway. 5% O_2 significantly increased the level of activated

Notch1 and their downstream gene, *HES1*. Furthermore, 5% O_2 markedly increased glucose consumption and lactate production of hADMPCs, which were decreased to normoxic levels by the treatment of γ-secretase inhibitor. In addition, we also found that HES1 was involved in the induction of GLUT3 expression through NF-κB signaling. These results clearly suggest that Notch signaling regulates glycolysis under hypoxic condition, and besides, likely affects the cell lifespan via glycolysis. These observations thus provide the new regulatory mechanisms of stemness maintenance obtained in 5% oxygen conditions. In addition, our study shed new light on the regulation of replicative senescence that would have impact for the quality control of hADMPC preparations used for therapeutic application.

T-3104

AMINOACYL-TRNA SYNTHETASE-INTERACTING MULTIFUNCTIONAL PROTEIN-3 IS A NOVEL REGULATOR OF THE NORMAL AGING PROCESSES OF STEM CELLS

Seunghee Lee, Young-Sil Ryu, Kyung-Rok Yu, Hyung-Sik Kim, Kyung-Sun Kang
Adult stem cell research center, Seoul, Korea, Republic of

Aminoacyl-tRNA synthetase-interacting multifunctional protein-3 (AIMP3/p18) is a member of the aminoacyl tRNA synthetase complex for protein translation. Recently, a senescence-related function of AIMP3 has been reported; however, the role of AIMP3 in normal aging processes has not been described. In this study, we observed an increase of AIMP3 in senescent human mesenchymal stem cells (MSCs) and aged mice-derived MSCs. The enforced expression of AIMP3 induced senescence phenotypes and these changes were reverted by AIMP3 inhibition. The same patterns were observed in Aimp3 transgenic- or hetero knockout mice. Moreover, the protein level of $p16^{INK4A}$, a representative senescence marker, was regulated by AIMP3. To identify the upstream regulators of AIMP3 during senescence processes, we screened possible epigenetic regulators and found that only miR-543 and miR-590-3p were significantly reduced in senescence-inducing conditions where AIMP3 protein levels were increased. Indeed, direct binding of these miRNAs to 3'UTR of AIMP transcripts was confirmed by 3'UTR-luciferase reporter assay. Taken together, AIMP3 is a novel regulator of the normal aging processes of adult stem cells and regulated by miR-543 and miR-590-3p directly in these processes.

T-3105

INTRAGLANDULAR TRANSPLANTATION OF BONE MARROW-DERIVED CLONAL MESENCHYMAL STEM CELLS CAN AMELIORATE POST-IRRADIATION SALIVARY GLAND HYPOFUNCTION

TacGhee Yi[1], Jae-Yol Lim[2], Jeong-Seok Choi[2], Songyi Lee[1], Yun Ho Jang[2], Hun Jung Kim[3], Young-Mo Kim[2], **Sun U. Song**[1]
[A]Translational Research Center, [B]Dept. of Otorhinolaryngology-Head and Neck Surgery, [C]Dept. of Radiation Oncology, [1]Inha University School of Medicine, Incheon, Korea, Republic of

External irradiation in head and neck cancers may induce irreversible hyposalivation and a consequent symptom, xerostomia, due to radiation damage to salivary glands (SGs). Cell-based therapy has been reported to repair or restore the damaged SG tissues. We tried to determine whether bone marrow-derived clonal mesenchymal stem cells (BM-cMSCs) can ameliorate irradiation-induced salivary hypofunction in a murine model. External irradiation at a dose of 15 Gy was carried out on the neck fields of C57BL/6 mice. We directly administered either homologous mouse BM-cMSCs, which were isolated by subfractionation culturing method, or PBS into the SGs 24 hours after irradiation. Salivary flow rate (SFR) and lag time of salivation were measured at 12 weeks after transplantation. SFR was significantly increased in BM-cMSCs-transplanted mice compared with PBS-injected mice. Histological evaluation at 12 weeks after BM-cMSC transplantation showed relatively well preserved acinar cells with normal morphology; however, damaged acinar cells with abnormal cytoplasmic vacuoles and aberrant nuclei were observed in PBS-injected control group. Less apoptotic cells and increased microvessel density were evident in BM-cMSC-transplanted SGs compared with PBS-injected SGs. Next we investigated whether locally injected BM-cMSCs into

the irradiated SGs survive and transdifferentiate into salivary epithelial cells. BM-cMSCs were labeled with fluorescent PKH26 and then injected into irradiated SGs. PKH26-labeled BM-cMSCs were detected in transplanted SGs at 4 weeks after transplantation. Furthermore, some PKH26-positive BM-cMSCs found within acino-ductal structures were demonstrated to express amylase by immunofluorescent staining, indicating transdifferentiation of BM-cMSCs into salivary acinar cells producing amylase *in vivo*. Our findings in this study suggest that highly homologous BM-cMSC can be used as a source of cell-based therapy for restoration of radiation-induced SG hypofunction.

T-3106

AUTOGENOUS POINT OF CARE BONE MARROW CONCENTRATE (BMC) FOR THE TREATMENT OF CERVICAL DEGENERATIVE DISC DISEASE IN SELF-PAY PATIENTS

Matthew Pouliot[1], Kenneth Pettine[2]

[1]The Orthopedic Stem Cell Institute, Loveland, CO, United States, [2]The Orthopedic Stem Cell Institute, Johnstown, CO, United States

Current
operative treatment options for moderate to severe symptomatic cervical
degenerative disc disease include fusion versus non-fusion technology. The use of autogenous BMC may provide a non-surgical option for this condition.
This is the very first report of the safety and efficacy utilizing
autogenous BMC for the treatment of cervical degenerative disc disease.
Purpose:
The
purpose of this study is to evaluate the safety and efficacy of autogenous BMC
for the non-surgical treatment of moderate to severe cervical degenerative disc
disease.
Study
Design:
This
is a prospective non-randomized study representing class II data.
Patient
Sample:
Thirty-two
patients (16 females, 16 males) were prospectively injected with autogenous BMC
into 66 cervical discs. The average age
was 53 with an average BMI of 26.2.
Outcome
Measures:
Every
patient had a pre-procedure neck disability index (NDI), visual analog scale
(VAS), physical examination, and MRI scanning.
The patients were followed prospectively at six weeks, three months, and
six months with repeat MRI scanning at six months.
Methods:
The
procedure takes 30 minutes and consists of IV sedation utilizing Versed and IV
Fentanyl. Percutaneous aspiration of the posterior iliac wing is performed to
obtain 60ml of bone marrow aspirate followed by concentration utilizing the
Spine Smith ART-21 system. Depending
upon the number of cervical discs to be injected, the volume of BMC was about
1ml. Typically, 0.5ml of BMC was

injected into each symptomatic cervical nucleus with a standard anterolateral approach to the disc with a 22-gauge spinal needle. The symptomatic discs were diagnosed based on MRI scanning.
Results:
The average pre-procedure NDI was 43.6% which improved to 23.8% at six months (p-value < 0.001). The pre-procedure VAS was 54 which improved to 25 at six months (p-value < 0.0001). At up to one-year follow-up in this group, no patient has undergone surgery. There was one discitis treated with antibiotics.
Conclusion:
These preliminary results utilizing autogenous BMC in a prospective study in 32 patients with minimum six-month follow-up indicate safety and very statistical efficacy. These six-month follow-up results indicate autogenous BMC has clinical efficacy for the non-operative treatment of degenerative disc disease in the cervical spine. These patients will continue to be followed for a minimum of two years.

T-3107

OLIGOPEPTIDE GRAFTED POLY(VINYL ALCOHOL CO ITACONIC ACID) SURFACE SHOWS ELASTICITY DEPENDENT MAINTENANCE OF PLURIPOTENCY IN HUMAN ADIPOSE TISSUE DERIVED STEM CELLS

Meng-Hsueh Wu, Ta-Chun Kao, Li-Yu Chen, Akon Higuchi
Department of Chemical Engineering and Material Sciences, National Central University, Jhongli, Taiwan

Stem cells purified from human adipose tissue (hADSCs) via serial culture of stromal vascular fraction (SVF) on tissue culture plates show multilineage differentiation ability in vitro. In the past decades, extracellular matrices (ECMs) such as collagen, fibronectin, vitronectin, and laminin were used as coating or self-standing materials on 2D culture and 3D culture of stem cells, and the differentiation and proliferation ability of stem cells (e.g., ADSCs or bone marrow-derived mesenchymal stem cells) were investigated. However, the origin of these ECMs are mainly animal-derived or human recombinant, and, therefore, it is not suitable to use them for clinical applications due to xeno-origin contamination and high cost of ECMs. On the other hand, it has been shown that physical environmental factors, such as matrix elasticity, and small functional groups including oligopeptides can have significant effect in determining stem cell fate. To achieve our ultimate goal of affordable, personalized regenerative medicine, clinically safe and economical ways to proliferate stem cells while maintaining their pluripotency as well as directing stem cell lineage specification without the use of induction chemicals are absolutely essential. In this study, we grafted several ECM-derived cell-adhesion peptides (KGGPQVTRGDVFTMP [cell-binding domain derived from vitronectin, oligoVN], KGGNGEPRGDTYRAY [cell-binding domain from bone sialoprotein, oligoBSP], and GKKQRFRHRNKG [heparin-binding domain, oligoHBD]) on polyvinylalcohol-co-itaconic acid (PVA-IA) hydrogels where the elasticity of PVA-IA gels can be changed from 100 kPa to 1 6MPa by regulating of crosslinking time. The pluripotency and differentiation ability were evaluated from culture of hADSCs on oligopeptide-grafted PVA-IA gels. Pluripotent genes, Sox2 and Nanog were extensively maintained on oligoVN and oligoBSP grafted PVA-IA gels having moderate elasticity around 2.6 MPa during one to five passages from qRT-PCR measurements, while Sox2 and Nanog expression of hADSCs cultured on conventional polystyrene tissue culture dishes (TCPS, 12 GPa elasticity) dramatically decreased with increasing passage numbers from one to five. We found that there is optimal elasticity of cell culture matrix to maintain pluripotency of hADSCs in this study. We evaluated osteogenic and chondrogenic differentiation ability of hADSCs where hADSCs were cultured on oligoVN, oligoBSP, and oligoHBD grafted PVA-IA gels having different elasticity and TCPS in each induction media at passage 6. Alizarin red staining (calcium deposition) and von Kossa staining (calcium phosphate deposition) indicated that hADSCs cultured on oligoVN and oligoBSP grafted PVA-IA gels having stiff elasticity (16 MPa) showed osteogenic differentiation with high efficiency

(more than 89%). It is concluded that both physical cue (elasticity of matrix) and biological cue (cell binding oligopeptides) are important factor to maintain stem cell pluripotency and to differentiate into specific lineages with high efficiency.

Muscle Cells

T-3111

THE IMMUNE RESPONSE TO IPSC-DERIVED MESOANGIOBLASTS IN VIVO

Ou Li[1], Joanna Hester[1], Giulio Cossu[2], Francesco Saverio Tedesco[2], Kathryn Wood[1]
[1]Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom, [2]University College London, London, United Kingdom

Allogeneic human pericyte-derived mesoangioblasts (muscle stem/progenitor cells) are currently in a phase I/II clinical trial for the treatment of patients with Duchenne muscular dystrophy, a severe neuromuscular disorder with childhood onset. However, limitations associated with the finite life span of these cells combined with the significant numbers of mesoangioblasts required to treat all of the skeletal muscles in these patients restricts their therapeutic potential. Induced pluripotent stem cell (iPSC)-derived mesoangioblasts may provide a solution to this problem. Although, the concept of using iPSC-derived cell therapies has been proposed for quite some time, our understanding of how the immune system interacts with these cells is inadequate. We have shown that iPSC-derived mesoangioblasts (HIDEMs) from healthy donors and, importantly, dystrophic patients can exert immunosuppressive effects on the proliferation of allogeneic T cells *in vitro*. Using a humanized mouse model, we have investigated the interaction between the immune system and HIDEMs *in vivo*. Although non-immunogenic in vitro, HIDEMs were targeted by allogeneic human T cells when transplanted beneath the kidney capsule in mice already reconstituted with human leukocytes; a process likely due to a Th1 mediated response. However, the immune response to the HIDEMs when they were already present as reconstitution with human leukocytes was taking place. We hypothesize that an immunosuppressive microenvironment needs to be established by the HIDEMs prior to the contact with allogeneic human T cells to enable the cells to exert their immunosuppressive function and to prevent from being targeted by T cells. We also showed regulatory T cells could reduce the targeting responses to HIDEMs and enhance their survival post transplantation. These data may provide useful insights to advance the clinical application potential of iPSCs.

T-3112

MYOD AND MYF5 HAVE ESSENTIAL BUT OVERLAPPING FUNCTIONS IN ADULT MYOGENESIS

Nicholas P. Legendre, Shoko YAMAMOTO, Masakazu Yamamoto, **David J. Goldhamer**
MCB, University of Connecticut, Storrs, CT, United States

The functions of the muscle regulatory factors (MRFs), MyoD and Myf5, in postnatal myogenesis have not been fully examined due to perinatal lethality of double-null mice. Mutations in either MyoD or Myf5 result in relatively minor defects, although it is unclear whether this reflects functional overlap between these MRFs, as in the embryo, or regulation of postnatal myogenesis by alternative pathways. We developed a MyoD conditional knockout allele, MyoDCKO, and analyzed the regenerative response of mice having different gene doses of MyoD and Myf5 using either the constitutive or satellite cell-specific Cre deleters CAG-CreERT or Pax7CreER, respectively. Tamoxifen-dependent knockout of the MyoDCKO allele in Myf5-/-;MyoDCKO/- mice resulted in severe and persistent defects in cardiotoxin-induced regeneration of the tibialis anterior muscle, and the size of the injured muscle remained dramatically reduced relative to controls through 40 days post-injury. In contrast, muscles having at least one wild type allele of MyoD regenerated normally. Histological analysis of injured, double-null muscles revealed the near complete absence of nascent myofibers; satellite cell progeny accumulated in injured muscles and were apparently incapable of forming nascent fibers. These muscles were filled with large adipocytes of non-satellite cell origin and exhibited pronounced hypercellularity. These data indicate that MyoD and Myf5 are required for satel-

lite cell differentiation, a conclusion supported by the inability of double knockout satellite cells to differentiate in culture. The roles of MyoD and Myf-5 in satellite cell programming are currently under investigation.

T-3113

THERAPEUTIC EFFECTS OF EXON SKIPPING AND LOSARTAN ON SKELETAL MUSCLE OF MDX MICE

Eun-Joo Lee, Ah-Young Kim, Eun-Mi Lee, Myeong-Mi Lee, Chang-Woo Min, Kyung-Ku Kang, Meeyul Hwang, Kyu-Shik Jeong
Department of pathology, Kyungpook National University, Daegu, Korea, Republic of

Duchenne muscular dystrophy (DMD) is characterized by the lack of dystrophin protein. Various attempts have been made to find innovative treatments for DMD patients. Exon skipping is one of the promising technologies for DMD treatment. However, exon skipping still has some limitations ; low efficiency and transitory treatment effects. It is well known that losartan, an angiotensinII type1 receptor blocker, promotes muscle regeneration and differentiation by lowering the transforming growth factor-β1 (TGF-β1) level. We hypothesized that the administration of losartan improves the exon skipping efficiency and possibly reduces the necessary repetitive treatments of exon skipping.
Mdx mice, the animal model of DMD, were used in this study. For exon skipping, 8mg/kg of phosphorodiamidate morpholino oligomer (vivo-morpholino, PMO) were injected into the tail vein. To induce muscle damage, mdx mice swam 3 times a week for 2 weeks. The mdx mice were divided into 4 groups : (1) mdx with swimming (S), (2) mdx with exon skipping after swimming (SP), (3) mdx with losartan administration (SL), (4) mdx with exon skipping after swimming and losartan administration (SLP).
More dystrophin was detected by RT-PCR and immune-fluorescence in the SP group, compared to the SLP. It is well known that PMO easily penetrates weak and injured muscles. For this reason, the SP group restored more dystrophin. The SP group had lots of weak muscle fibers due to the continuous mechanical injury. However, the SLP group had less weak muscle fibers because of the muscle regeneration effects of the losartan. Because of this, the SP group showed a higher exon skipping efficiency than the SLP group.
The SLP group showed less centronucleated muscle fibers and calcification area than that of the SP group. To validate muscle regeneration, the expression level of Pax7, Myf5, MyoD and Myogenin were examined by qRT-PCR. All four genes were up-regulated in the SP group, compared to S. However, the expression level of the four genes showed little difference between the SLP and SL groups. These results indicate that SLP has already completed muscle regeneration with the help of losartan and restored dystrophin by exon skipping. It is well-known that restored dystrophin helps to improve muscle function even at a small quantity of dystrophin. It seemed that the restored dystrophin in the SLP group was enough to recover the normal structure of muscles even though it was a lower level than that of the SP group.
Exon skipping without losartan is more effective than exon skipping with losartan. Otherwise, exon skipping with losartan promoted muscle regeneration and shortened the time required to restore normal muscle structure. We demonstrated that proper niche is important in muscle regeneration and DMD treatment. In conclusion, we purposed a new concept to overcome DMD by the combined therapy of exon skipping and losartan administration.

T-3114

T CELL MEDIATED INFLAMMATION IS REQUIRED FOR MUSCLE STEM CELL PROLIFERATION IN MUSCLE REGENERATION

Xin Fu, Jun Xiao, Sheng Li, Hengjiang Guo, Hongyan Wang, Ping Hu
Shanghai Institute of Biochemistry and Cell Biology, CAS, Shanghai, China

Skeletal muscle regeneration involves a series of physical responses after injury or disease, including activation of quiescent satellite cells (muscle stem cells), proliferation of satellite cells and myoblasts, differentiation of myoblasts, and formation of new myofibers. In recent years, more and more evidences suggested that inflammation

plays important roles during muscle regeneration process. However, how inflammation affects muscle regeneration remains to be elusive.
Here we focused on T cells mediated inflammation and found that it is a required positive regulator at early stage of skeletal muscle regeneration. Upon muscle injury, we observed large amount of T cell infiltrated at injury site. In immunodeficient mice, where the T cell infiltration is diminished while other lymphocytes such as macrophage infiltration remains normal, reparation of muscle injury was dramatically delayed. To further investigate the mechanism of T cell promoting muscle regeneration, we characterized the protein profile of activated T cells. A combination of four factors was identified to be able to promote satellite cell proliferation and long term expansion dramatically in culture. The cultured expanded satellite cells continue to express muscle stem cell marker, and were able to regenerate functional myofibers in vivo. Furthermore, muscular injection of the four factor cocktail could rescue the muscle regeneration defects caused by T cell deficiency. Our results demonstrate that T cell mediated inflammation is required for muscle stem cell proliferation at early stage of post-injury regeneration.

T-3115

REVERSIBLE CELL IMMORTALIZATION ALLOWS HAC-MEDIATED GENE CORRECTION OF HUMAN DYSTROPHIC MYOGENIC PROGENITORS

Sara Benedetti[1], Hidetoshi Hoshiya[1], Martina Ragazzi[1], Yasuhiro Kazuki[2], Mitsuo Oshimura[2], Graziella Messina[3], Giulio Cossu[1], **Francesco Saverio Tedesco**[1]
[1]Department of Cell and Developmental Biology, University College London, London, United Kingdom, [2]Department of Biomedical Science, Tottori University, Yonago, Japan, [3]Department of Biology, University of Milan, Milan, Italy

Duchenne muscular dystrophy (DMD) is caused by mutations in the dystrophin gene and affects skeletal muscles, resulting in premature death. There is no treatment, but new strategies are under investigation. Among these, gene and cell therapy is complex, since dystrophin is the largest human gene (2.4Mb). In recent years donor mesoangioblast (vessel-associated muscle stem/progenitor cells) transplantation caused amelioration of DMD animal models. Moreover, these cells are under clinical experimentation for DMD in a phase I/II trial. However, autologous transplantation would be desirable, since it would not require immune-suppression. To this end, we previously reported the amelioration of DMD mice by combining Human Artificial Chromosome (HAC)-mediated dystrophin gene-replacement with mesoangioblast transplantation (using a HAC containing the entire dystrophin locus: DYS-HAC). Nevertheless, in order to translate this strategy to human mesoangioblasts their proliferative capacity needs to be extended to ensure survival after HAC transfer. Here we describe the reversible immortalization of mesoangioblasts using lentiviral vectors encoding floxed hTERT-IRES-HSV1-TK (human telomerase and herpes simplex virus thymidine kinase) and Bmi-1 to bypass replicative senescence. Mesoangioblasts have been characterized for proliferation and expression of hTERT and Bmi-1 and remained growth factor-dependent, contact-inhibited, not tumorigenic and myogenic. Notably, this strategy allowed the transfer of a novel DYS-HAC (DYS-HAC2) with reduced immunogenicity into reversibly immortalized DMD mesoangioblasts. Clones have been screened and expanded, showing normal karyotype and episomal maintenance of the DYS-HAC2. Importantly, upon transplantation in dystrophic mice, reversibly immortalized DMD mesoangioblasts corrected with the DYS-HAC2 were integrated into regenerating myofibers and correctly produced human dystrophin. These data provide relevant information for muscle stem cell biology, chromosome engineering and future therapeutic strategies for muscular dystrophies.

T-3116

TARGETING ENDOTHELIAL JUNCTIONAL ADHESION MOLECULE-A/ EPAC/ RAP-1 AXIS AS A NOVEL STRATEGY TO INCREASE STEM-CELL ENGRAFTMENT IN MURINE DYSTROPHIC MUSCLES

Sara Benedetti[1], Monica Giannotta[2], Francesco Saverio Tedesco[1], Monica Corada[2], Rocco D'Antuono[2], Beatriz G. Galvez[3], Giulio Cossu[1], Elisabetta Dejana[2]

[1]Cell and Developmental Biology, University College London, London, United Kingdom, [2]IFOM, FIRC Institute of Molecular Oncology Foundation, Milan, Italy, [3]National Centre for Cardiovascular Research, Madrid, Spain

Muscular dystrophies are a group of genetic diseases characterized by severe and progressive muscle wasting. Currently no definitive treatments exist, although a number of experimental strategies have been developed and brought into clinical experimentation. Among these cell therapy holds great promises and potential. However, one of main limitations of the stem cell-based strategies is the limited engraftment of cells to the diseased tissue. Some years ago, mesoangioblasts were isolated as vessel-associated myogenic stem/progenitor cells able to cross the vessel-wall and to contribute to muscle regeneration after intra-arterial administration. Although donor mesoangioblasts are undergoing clinical experimentation for Duchenne muscular dystrophy, mechanisms and molecules regulating extravasation still need to be elucidated. In order to study and understand this process we took advantage of insights from leukocyte and vascular biology. Specifically, we focused our attention on JAM-A, an endothelial junctional protein regulating leukocytes migration into the inflamed tissues. Here, we demonstrate that the abrogation of endothelial JAM-A expression or function strongly enhances murine mesoangioblast engraftment and differentiation into dystrophic and acutely injured muscle. This has also been confirmed into "ad hoc"-generated double-mutant dystrophic alpha-sarcoglycan-null/JAM-A-null mice. Notably, in vitro assays showed that JAM-A inhibition reproducibly improved also human mesoangioblast trans-migration, suggesting relevance for this strategy for future clinical translation. Moreover, we found that in the absence of JAM-A, the exchange factors EPAC-1 and 2 are down-regulated, thus preventing the activation of Rap-1, a small GTPase protein involved in cell adhesion and junction formation. As a result of impaired Rap-1 activation, junction tightening is reduced, therefore facilitating cell extravasation. Remarkably, pharmacological inhibition of Rap-1 also increases cell engraftment into dystrophic muscle. These data provide important mechanistic insights on muscle stem/progenitor cell engraftment and are relevant for improving efficacy of cell-based therapies for muscular dystrophies.

T-3117

ROLE OF P53 AND OF ITS DOWNSTREAM EFFECTOR P21 IN SKELETAL MUSCLE STEM CELL SENESCENCE

Francesca Boscolo Sesillo, Alessandra Sacco
Sanford-Burnham Medical Research Institute, La Jolla, CA, United States

Duchenne Muscular Dystrophy (DMD) is a progressive skeletal muscle degenerative disease caused by mutations in the dystrophin gene. Dystrophin is a protein responsible for linking the cytoskeleton of the myofibers to the extracellular basal lamina and its absence induces fragility of the sarcolemma. As a result, DMD skeletal muscles undergo continuous cycles of damage and regeneration, which is mediated by endogenous skeletal muscle stem cells (MuSCs). Over time, this repair process becomes inefficient and DMD muscle tissues exhibit signs of premature aging. Indeed, previous studies have shown that tissue samples from DMD patients display shorter telomeres compared to age-matched healthy controls. Consistent with these findings, our previous work demonstrated that crossing the dystrophic mdx mouse with animals lacking telomerase activity generates a model, mdx/mTR that more closely recapitulates the severe DMD phenotype observed in humans. Critical telomere shortening has been associated with aging of several tissues. This biological process induces the DNA repair pathway through activation of the tumor suppressor protein p53. We hypothesize that activation of p53, triggered by telomere shortening, plays a role, in the premature MuSCs senescence and reduced repair function observed in dystrophic muscles. To test this hypothesis we generated two new mouse models: mdx/mTR mice in either p53 or p21 null background.
Preliminary results obtained from histological analysis and serum creatine kinase activity measurements, indicated no muscular damage in control p53 and p21 single knockout mice. Interestingly, we observed increased variability in myofiber size in mdx/mTR mice heterozygous for either p53 or p21 after first generation breeding. This finding suggests that dystrophic mdx/mTR mice, heterozygous for either p53 or p21, are going through the process of regeneration more efficiently than control counterparts. MuSC number, identified by Pax7 expression, was comparable among samples. Observed differences in fiber size and histological profile associated with similar number of MuSCs suggest that the behavior of this cell type in the muscle is dissimilar and needs to be further evaluated with MuSCs isolation. Preliminary data indicate that MuSCs isolated from dystrophic mdx/mTR/p53-/- mice exhibit an

increased proliferation rate compared to dystrophic controls with intact p53. These findings suggest that p53 and p21 may participate in MuSC senescence and p53 activation may be a consequence of critical telomere shortening and DNA damage. Overall, these studies will elucidate the role of this stress-activated pathway in premature senescence in dystrophic MuSCs and aging of skeletal muscles.

T-3118

HESC-SECRETED FACTORS ENHANCE HUMAN MYOBLAST PROLIFERATION AND MUSCLE REGENERATION BY MODULATING THE MAP KINASE, NOTCH, AND WNT SIGNALING PATHWAYS

Hanadie Yousef[1], Mike Conboy[2], Hikaru Mamiya[2], Matthew Zeiderman[1], David Schaffer[2], Irina Conboy[2]
[A]Molecular and Cell Biology, [B]Bioengineering, [1]UC Berkeley, Berkeley, CA, United States

Adult stem cells typically grow relatively poorly as compared with embryonic cells of the same tissue type, and stem cell maintenance and proliferation progressively declines with age. We postulated that the embryonic niche environment generally supports robust proliferation and maintenance of tissue specific stem cells and tested this hypothesis on muscle progenitor cells (primary myoblasts). hESC-conditioned medium robustly enhanced the proliferation of both human and mouse myoblasts, even in pro-differentiation conditions. Factors in the conditioned medium were confirmed to be proteins secreted by hESCs. A proteomics array comparing hESC-conditioned medium with differentiated cell-conditioned medium lacking the pro-proliferative effect showed that multiple FGFs are enriched in the hESC-conditioned medium. The FGFs showed a dose-dependent enhancement of myoblast proliferation and reduced differentiation, and were dependent on the MAP Kinase pathway. Further mechanistic analysis showed that hESC-secreted factors enhance MAPK and Notch signaling pathways, and that Notch signaling only increases when MAPK signaling is induced, suggesting cross-talk between the two pathways. hESC-conditioned medium also increased downstream Wnt signaling in a MapK independent manner. Together these results support that multiple pathways coordinate a young and growing tissue stem cell environment.

T-3121

HUC-MSC (HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELL) RECOVER THE MUSCLE REGENERATION IN ANTIGRAVITY MUSCLE INDUCED HINBLIMB SUSPENSION RAT

Si-Hyun Heo
CHA Univ, Gyeonggi-do, Korea, Republic of

Research about space life science has been actively conducted, astronauts who exposed to long term state of nongravitation were faced physiological changes. Unique environment of space microgravity is known to cause muscular atrophy. Actually, the astronauts exposed to the space environment throughout the whole body are induced muscle atrophy. Although many treatments such as electrical stimulation, treadmill training for the treatment of muscle atrophy are in use, have lots of limitations. MSC (mesenchymal stem cell) has been used in the treatment of many incurable diseases, we compared the efficacy of various type of MSCs local transplantation for the treatment of muscular atrophy on hindlimb suspension model. Female Sprague Dawley rats induced hindlimb suspension, DiI fluorescent coated MSC ($1x10^6$cells) were injected into the soleus muscle on 14 days after hindlimb suspension. As a result, muscle mass was increased in MSC injected groups compared to control group, also promoted muscle regeneration. Histological analysis was more characterized during muscle regeneration, showed larger cross section area in the experimental groups. Muscle specific marker expression was higher in the MSC transplantation groups compared to control group. The present study demonstrated that MSCs transplantation, especially hUC-MSC, promoted muscle regeneration and inhibited muscular dystrophy. These results suggest that

intramuscular transplantation of hUC-MSC may be used in cell therapy of muscular atrophy for astronauts.

T-3122

PERICYTE HETEROGENEITY IN TISSUE REPAIR

Alexander Birbrair[1], Tan Zhang[1], Zhong-Min Wang[1], Maria Laura Messi[1], Grigori N. Enikolopov[2], Akiva Mintz[1], Osvaldo Delbono[1]

[1]Wake Forest University, Winston Salem, NC, United States, [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, United States

Normal tissue homeostasis as well as tissue regeneration and repair after damage rely on resident stem cells. Incomplete regeneration in mammals has been attributed to an insufficient number of stem cells and a rapid fibro/adipocytic infiltration after wounding. Excessive scar formation compromises tissue function and can lead to organ failure. Cells called pericytes, embedded in the basement membrane of capillaries, provide physical strength and nurturing signals to sprout new blood vessels. Some studies suggest that pericytes may contribute also to regeneration in various organs as well as scar formation in response to pathologies. Based on markers and morphology, pericytes have been identified as heterogeneous. However, their differentiation capability was not yet explored. Transplantation studies indicate that type-2 pericytes participate in muscle regeneration, while type-1 contribute to fibrous and adipose tissue accumulation. Additionally, only type-2 pericytes have the potential, under optimized culture conditions, to generate neural cells. We propose they can be used as an alternative to treat CNS pathologies that require cell replacement. Our results support that pericytes involved in tissue repair differ from those involved in scar formation after tissue injury.

T-3123

ENGINEERING BACTERIAL SECRETION SYSTEMS WITH TRANSDIFFERENTIATION POTENTIAL

Analise Z. Reeves[1], William E. Spears[1], Amy J. Wagers[2], Cammie F. Lesser[1]

[1]Department of Medicine, Division of Infectious Diseases, Massachusetts General Hospital, Cambridge, MA, United States, [2]Department of Stem Cell and Regenerative Biology, Harvard Stem Cell Institute, Cambridge, MA, United States

Many gram-negative bacteria utilize type 3 secretion systems (T3SSs) to deliver virulence proteins, called effectors, directly into host cells. The addition of an N-terminal type 3 secretion signal to heterologous proteins allows for recognition and secretion of proteins not typically recognized as T3SS substrates. We aim to utilize this property of T3SSs as a biological tool for applications requiring the directed delivery of proteins into mammalian cells. We have engineered non-pathogenic E.coli strains that encode the structural components of the Shigella flexneri type 3 secretion machinery on a low-copy plasmid. The addition of the first 50 amino acids of an effector to mammalian proteins is sufficient for secretion and delivery of heterologous proteins into mammalian cells. Experiments utilizing an E coli strain capable of secreting and delivering the myogenic transcription factor, MyoD, into mouse embryonic fibroblasts (MEFs) demonstrate that delivery of MyoD by the E. coli results in the induction of muscle-cell specific genes and ultimately drives their transdifferentiation into myosin heavy chain-expressing muscle cells, demonstrating that this approach can be used to alter the developmental fate of a cell. Ultimately, we seek to engineer similar E.coli strains that utilize T3SSs to deliver iPS reprogramming factors, enabling integration-free production of pluripotent stem cells for experimental and therapeutic applications.

Cardiac Cells

represent a renewable source of relevant cells to substitute for ex vivo material. hPSC-CMC have been extensively characterized and demonstrate a clear cardiac phenotype. Furthermore, the tissue-like format of hPSC-CMC offers an integrated system for studying the multiple signal transduction pathways involved in the hypertrophic response.

AIM: hPSC-CMC were assessed for use as a potential in vitro model for the study of PCH.
Method: hPSC-CMC were exposed to established inducers of cardiac hypertrophy, including endothelin 1, norepinephrine, and phenylephrine, under serum free conditions. Gene expression profiling was performed to confirm induction of the fetal gene re-programming typically associated with a hypertrophic response. Cluster volume, extrapolated from hPSC-CMC cross sectional area, served as indicator for increases in cell size. Markers for cell proliferation and cell death were used to evaluate the mechanisms behind hPSC-CMC morphological changes. Electrophysiological characteristics were examined with a multi electrode array system.
Results: Endothelin 1, norepinephrine, and phenylephrine increased atrial natriuretic peptide (ANF) and brain natriuretic peptide (BNP) gene expression, and altered the ratio of MYH6 to MYH7. Expression of sarcolipin, a regulator of the sarcoplasmic reticulum ATPase calcium pump, was significantly increased. The role of calcium in the hypertrophic response was further investigated with the calcium channel blockers verapamil and nifedipine. Inhibition of the calcium handling mechanisms depressed the response to the hypertrophic compounds.
Having established that the hypertrophic response was inducible in hPSC-CMC, further studies were performed to assess the effect of hypertrophic re-modeling on cardiomyocyte functionality. In particular, susceptibility of the hypertrophic hPSC-CMC to arrhythmia was examined.
Conclusions: Hypertrophic responses can be observed in hPSC-CMC suggesting that these cells have a great potential to serve as an integrated disease model, where multifactorial stimuli, such as cell to cell and extracellular matrix signaling, can be studied in a relevant cardiac population.

T-3133

MICRORNA-AUGMENTED DIFFERENTIATION OF CARDIOMYOCYTES FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Chani Hodonsky, Kaihong Wu, Lakshmi Mundada, Ming-Sing Si
Cardiac Surgery, University of Michigan, Ann Arbor, MI, United States

Introduction: The crucial role that microRNAs (miRNAs) play in the development, function and maintenance of every cell type has made it a target for cellular reprogramming. The potential effects on reprogramming cells into terminally differentiated cell types such as neurons and cardiomyocytes (CMs), which are incapable of post-injury proliferation and regeneration have recently been explored. However, current methods of direct reprogramming of fibroblasts into CMs using miRNAs is very inefficient.
Current methods of differentiating human induced pluripotent stem cells (iPSCs) into CMs are very effective in generating nearly pure populations. Although the appearance of contractile cells may appear as early as 7 days during these differentiation protocols, all require nearly 30 days of culture before the entire population of iPSCs become pure CMs. Increasing the rate of differentiation and maturation of these CMs would be important for eventual clinical application and scale-up. Because of the important role that miRNAs play in regulating wide networks of genes and their potential for direct reprogramming, we hypothesized that miRNAs known to play a role in cardiac disease and development can augment and accelerate the differentiation of iPSCs into CMs. Here we present results of our ongoing efforts to combine iPSC forward reprogramming with a CM differentiation protocol to explore this hypothesis.
Methods: We utilized an extracellular matrix (ECM) to promote the mesodermal specification and sequential exposure of iPSCs to activin A and BMP4 to promote CM differentiation. We are evaluating the potential of miRNA 1, 133, 208 and 499 alone or in various combinations to accelerate CM differentiation. miRNAs were embedded in the basal ECM layer as an efficient method of nontoxic and nonviral delivery. Techniques for evaluating successful differentiation have included immunofluorescence, qPCR of iPSC and CM specific genes and quantitative analysis of mature-CM-specific proteins.
Results: Using the combination of miRNAs previously described to cause direct reprogramming of murine fibroblasts into CM like cells_miRNAs 1, 133, 208, and 499_we have begun to analyze the effects of miRNAs on affecting the differentiation of iPSCs into CMs. The four-miRNA combination significantly increased the proliferation, but slowed the differentiation of human iPSCs. Cells with the four-miRNA combination had decreased CM specific gene expression as compared to controls that underwent differentiation without miRNAs. Our preliminary data also in-

dicate that different combinations of these miRNAs have varying effects on the speed of differentiation. For instance, we found that the combination of miRNA 1 and 499 accelerate the appearance of beating cells as compared to controls (no miRNAs).
Conclusions: Our preliminary results indicate that miRNA 1, 133, 208 and 499 actually delays the differentiation of human iPSCs into CMs. Unexpectedly, this four-miRNA combination significantly increased the proliferation rate of iPSCs. Other results indicated that a subset of the above miRNAs do accelerate iPSC differentiation into CMs, providing proof of principle that combining forward reprogramming of iPSCs with miRNAs and a CM differentiation protocol can accelerate the generation of CMs. Current work is being done to further define this strategy as well as characterize the maturity of these CMs that were the product of miRNA accelerated-differentiation from human iPSCs.

T-3134

STIMULATION OF CARDIOMYOCYTE REGENERATION IN HEART MUSCLE FROM HUMAN INFANTS

Stuart Walsh, Bernhard Kühn
Harvard Medical School, Boston Children's Hospital, Boston, MA, United States

The human heart has low regenerative capacity, and injury frequently results in organ damage, heart failure, and death. One therapeutic strategy currently under development is to stimulate endogenous regenerative processes. Proliferation of heart muscle cells, cardiomyocytes, is the underlying mechanism of effective myocardial regeneration in lower vertebrates and of myocardial growth in young humans. The objective of this study was to assess the ability to stimulate cardiomyocyte mitosis in heart muscle (myocardium) derived from patients with Tetralogy of Fallot (ToF), one of the most common types of congenital heart disease (CHD).
In patients with ToF (n = 21), in the age range of 2 months - 66 years of age, the percentage of Ki-67 and H3P-positive cardiomyocytes was decreased in comparison with age-matched un-diseased human hearts. To determine whether cardiomyocyte cell cycle activity can be rescued, we developed an organotypic culture system. Addition of Neuregulin-1 (NRG1), a growth factor known to increase cardiomyocyte cell cycle activity in animals, induced a 4-fold increase of the percentage of H3P-positive cardiomyocytes in myocardium from ToF-patients aged 2 to 5 months of age (n = 9). In contrast, NRG1 did not stimulate cardiomyocyte cycling in myocardium from patients > 6 months of age (n = 12), suggesting that the ability to stimulate cardiomyocyte cycling with NRG1 was lost at that age.
Cardiomyocytes from young ToF patients can be induced to proliferate with recombinant rNRG1. The ability to stimulate cell cycle activity of endogenous cardiomyocytes in human myocardium may provide a new strategy for the prevention and treatment of heart failure in young patients with CHD.

T-3135

INHIBITION OF MITOCHONDRIAL PERMEABILITY TRANSITION PORE AND TREATMENT OF ANTIOXIDANT PROMOTE CARDIOMYOCYTE DIFFERENTIATION FROM PLURIPOTENT STEM CELLS

Sung Woo Cho, Jin Sung Park, Gou Young Koh
Graduate School of Medical Science and Engineering, KAIST, Daejeon, Korea, Republic of

Efficient differentiation of cardiomyocytes (CMs) from pluripotent stem cell (PSC) is required for treating patients with cardiac diseases such as ischemic heart disease and severe congestive heart failure. Here, we showed that Cyclosporin A (CsA) promoted robust CMs differentiation from embryonic stem cell and induced PSC-derived Flk1+ mesodermal precursor cells (MPCs) in an OP9 co-culture system. CMs obtained by the treatment of CsA showed cardiac specific electrophysiological properties. The cardiomyogenic effect of CsA was mainly caused by mitochondrial permeability transition pore (mPTP) inhibition rather than calcineurin inhibition. The mPTP inhibitor NIM811 which does not have inhibitory effect of calcineurin promoted CMs differentiation as much as CsA, but calcineurin inhibitor FK506 slightly increased CMs differentiation. CsA induced differentiation of CMs appears to have resulted from activation of mitochondrial oxidative metabolism. CsA treated Flk1+ MPCs showed increase of mito-

chondrial membrane potential, mitochondrial calcium, reactive oxygen species, oxygen consumption rate and ATP level and change of gene expression related to mitochondrial oxidative metabolism and biogenesis. In addition, CMs obtained by the treatment of CsA showed more matured sarcomeres and mitochondria. We demonstrated that mPTP inducer, carboxyatractyloside reversed the cardiomyogenic effect of CsA. Also, treatment of antioxidant, 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) with CsA dramatically increased CM differentiation compared to CsA treatment alone. Therefore, our data showed that mPTP inhibition, mitochondrial oxidative metabolism and redox regulation is important for CMs differentiation and maturation from PSC.

T-3136

TRANSCRIPTOME PROFILING OF HUMAN INDUCED PLURIPOTENT STEM CELL DIFFERENTIATION INTO CARDIOMYOCYTES BY NEXT-GENERATION RNA SEQUENCING

Matthew J. Spindler[1], Yuichiro Miyaoka[1], Mohammad A. Mandegar[1], Nathaniel Huebsch[1], Luke M. Judge[1], Trieu Nguyen[1], Caitlin R. Russell[1], Paweena Lizarraga[1], Po-Lin So[1], Nathan Salomonis[2], Bruce R. Conklin[1]

[1]Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, United States, [2]California Pacific Medical Center Research Institute, San Francisco, CA, United States

Human development requires tight spatial and temporal control of signaling events and transcriptional regulation for the formation of all the body's tissues. Although valuable insights into signaling and transcriptional mechanisms necessary for normal organ and tissue development have been provided by developmental studies in mice, zebrafish, and Drosophila, extensive studies on human development have not been possible until recently. The discovery of human induced pluripotent stem (iPS) cell technology allows us to derive cell lines from individuals whose phenotypes can be readily assessed. Thus, iPS cells offer an ideal model system to understand human developmental processes and how these events could be altered in diseased conditions. We are using this system to finely map the transcriptional changes that occur during the differentiation of human iPS cells into cardiomyocytes (iPS-CMs) to better understand human cardiac development.
We hypothesize that coordinated gene expression changes and alternative mRNA processing events are required for the proper differentiation of iPS cells into iPS-CMs. We are using next-generation RNA sequencing (RNA-seq) to discover new transcriptional patterns that identify important processes during iPS-CM differentiation. This approach is expected to provide an unbiased, high resolution, picture of human cardiac development. To model normal cardiomyocyte development, we have optimized a differentiation protocol that uses Wnt signaling modulation (CHIR99021 & IWP4) to yield over 75% cardiac troponin positive cells from multiple integration free iPS cell lines. These normal iPS cell lines were derived from healthy volunteers that had normal EKGs and no known cardiomyopathy-associated mutations. We collected RNA during the course of iPS-CM differentiation and used quantitative PCR (qPCR) to assess the expression of known genes regulated during cardiac development. The expression patterns of these genes identified specific, sequential, steps of iPS cell differentiation into mesendoderm, cardiac mesoderm, cardiac progenitors, immature, and mature cardiomyocytes. To better understand the transcriptional changes that occur during iPS-CM differentiation, we are conducting RNA-seq on these specific steps to identify key and novel gene expression changes and alternative splicing events important for proper cardiac development. We predict that these studies, along with preliminary exon-level microarray analyses, will identify a number of transcriptional events critical for each step of iPS-CM differentiation. In addition, we expect these results to serve as a reference for normal human cardiomyocyte development and to inform disease models including congenital heart defects, cardiomyopathies, and arrhythmias related to Nkx2.5, SCN5A, RBM20, MYBPC3, PKP2, and BAG3.

T-3137

VCAM1 AND SIRPA CELL SURFACE MARKERS OF HUMAN CARDIOMYOCYTES

Rhys JP Skelton[1], Edouard G. Stanley[2], Andrew G. Elefanty[2], Ben W. Finnin[3], Katerina Koutsis[1], Deevina Arasaratnam[1], Anthony J. White[1], Colin W. Pouton[3], John M. Haynes[3], Reza Ardehali[4], David A. Elliott[1]

[A]Cardiac Development Laboratory, [B]Stem Cell Laboratory, [1]Murdoch Children Research Institute, Melbourne, Australia, [2]Cardiac Development Laboratory, Monash Pharmacology, Melbourne, Australia, [3]Eli and Edith Broad Stem Cell Research Center, Murdoch Children Research Institute, Los Angeles, CA, United States

Heart disease is the leading cause of mortality and morbidity in the Western world. The human heart has a limited regenerative capacity and there is an unmet demand for improved therapies for myocardial disease. Human stem cell derived cardiomyocytes have potential to facilitate the development of both cell based and pharmaceutical treatments. To date many studies have focused on the efficient generation of cardiomyocytes from pluripotent hESCs and iPSCs. Efficient *in vitro* differentiation of human cardiomyocytes is becoming a standard technique and hESC derived cardiomyocytes are beginning to be used in cardiotoxicity screens. In order to capitalise on these advances in cardiomyocyte generation and ensure the transfer of this technology into biopharmaceutical and clinical settings, enriched and well defined cell populations are required. Therefore, cell surface markers that allow the facile purification of cardiac cell lineages are needed.

This work investigates the potential of two novel cell surface markers, SIRPA and VCAM1, in isolating enriched populations of committed human cardiomyocytes. The NKX2-5GFP/w reporter line was used to characterise the temporal expression, gene expression profiles and functional properties of SIRPA and VCAM1 cell populations that are found during cardiac differentiation. We demonstrate that fully functional cardiomyocytes arise from an NKX2-5^{+}SIRPA^{+} intermediate which up regulates VCAM1 concomitantly with commitment to the myogenic lineages of the heart (ie. smooth and cardiac muscle). Under the culture conditions used in this study, the NKX2-5^{+}SIRPA^{+} population had a higher single cell clonogenic potential and displayed the capacity to give rise to both smooth muscle and cardiac clones. Furthermore, when cultured as re-aggregates the majority of NKX2-5^{+}SIRPA^{+} cells progressed to a NKX2-5^{+}SIRPA^{+}VCAM1^{+} phenotype thus demonstrating lineage relationship of these two cell populations.

The identification of these cell surface markers provide a means for further study of linage specification during in vitro human cardiogenesis and will facilitate the development of technologies to produce well-characterised, purified human cardiomyocytes.

T-3138

FUNCTIONAL ALTERATIONS IN HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTE DUE TO IN VITRO CULTURE AND REVERSING EFFECT OF VITAMIN C

Yoon Young Kim, Seung-Yup Ku, Seok Hyun Kim, Young Min Choi, Shin Yong Moon
IRMP, MRC, Seoul Natl University, Seoul, Korea, Republic of

Human embryonic stem cells (hPSCs) have capacities to self-renew indefinitely and differentiate into all the cells in the body including cardiomyocyte. This property of hPSC-derived CM makes them considered as an ideal model for various studies. Aging of CM is found in natural human cardiomyocyte, however, due to limitation in use of human cells, hPSC-derived CM could be a model for aging of CMs. In this study, we tried to demonstrate the functional alteration in hPSC-derived CMs and reversing effect of vitamin C. Human pluripotent stem cell-derived CMs are classified due to in vitro cultivation period as early-, middle- and late-stage.

Through SA β-gal staining, alterations in hPSC-derived CMs of middle- and late-stage were evaluated and to reverse the alteration, various concentrations (0, 100, 250 μM) of vitamin C was treated for 0, 24, 48 hrs during culture. The functionality of hPSC-derived CMs was evaluated by JC-1 staining, the specific dye for mitochondrial membrane potential.

Middle and late-stage hPSC-derived CM showed increased number of pigmented cells and decreased beating rate in correlation with *in vitro* culture period. Positively-stained cells of SA-β-gal staining were abundant in late-stage hPSC-derived CMs. Treatment of vitamin C significantly reduced the portion of positively-stained cells at each stage and affected largely in late stage cells. Positive population of JC-1 staining was correlated to the alteration of hPSC-derived CMs.

In this study, we tried to find out the functional alterations of hPSC-derived CM and proved the reverse effect of vitamin C. These results suggested the possible usage of them for further studies related to aging of human cardiomyocyte (2012-0004131 and A111539).

T-3141

CONSTRUCTING A NATIVE ENVIRONMENT FOR THE DIFFERENTIATION OF IPSCS TO CARDIOMYOCYTES.

Chani J. Hodonsky, **Kaihong Wu**, Ming-Sing Si
Cardiac Surgery, University of Michigan, Ann Arbor, MI, United States

Introduction: The generation of mature differentiated cells from iPSCs for clinical therapies has widespread implications, especially regarding diseases involving terminally differentiated cells such as cardiomyocytes, which are incapable of post-injury proliferation and regeneration. The optimal protocol for cardiomyocyte differentiation via iPSCs has been under study for several years. However, the length of the protocol (up to two months, beginning with autologous somatic cells from which iPSCs are derived) as well as some of the techniques involved (viral transduction of transcription factors) make the current protocols impractical for clinical use.
We aim to improve on current protocols for differentiation of human iPSCs into cardiomyocytes. Several studies have implicated physical environment in the fate of differentiating cells. Furthermore, the roles of extracellular growth factors and paracrine factors have long been known to affect differentiation outcome. It can be speculated that the ideal environment for an iPSC differentiating into a cardiomyocyte would be the native environment of all differentiating cardiomyocytes. For these reasons, we plan to evaluate the addition of decellularized cardiac extracellular matrix (ECM) to our current differentiation protocol to reduce the length of the current protocol. We propose that the addition or substitution of ECM for the top layer of Matrigel will expose the differentiating cells to factors that can be found in the native cardiac environment during development, therefore increasing their potential to differentiate into mature cardiomyocytes, an outcome that has not been achieved by previous studies.
Methods: Our research focuses on forward-differentiating iPSCs into cardiomyocytes using a Matrigel sandwich and added growth factors, as described by Zhang et al. Techniques for evaluating successful differentiation will include immunofluorescence, quantitative PCR of stem-cell-specific as well as cardiac-specific transcripts, and quantitative analysis of mature-cardiomyocyte-specific proteins.
Results: Preliminary data indicate that ECM in addition to all other components of our current protocol is insufficient to accelerate proliferation or differentiation of iPSCs compared to controls. This is possibly due to toxicity from high levels of the reagents involved; an alternative explanation is that the combination of Matrigel and ECM reduces the efficiency of each one separately. Therefore, we plan to augment our protocol by substituting varying levels of ECM for other structural components of the protocol. When compared to control iPSCs, we expect ECM-treated cells to display a phenotype and expression of cardiac-specific transcription factors and structural proteins closer to adult human cardiomyocytes.
Our aim is to generate cardiomyocytes from an autologous source that could be used as an alternative to current heart-failure therapies. The successful production of viable, mature cardiomyocytes for human patient use would be a boon for treating cardiovascular disease.

T-3142

HUMAN iPSC-DERIVED CARDIOMYOCYTES PROVIDE A RELEVANT MODEL OF CARDIAC HYPERTROPHY FOR USE IN PHENOTYPIC SCREENING AND DRUG DISCOVERY

Blake Anson[1], Coby Carlson[2], Chad Koonce[3], Shannon Einhorn[2], Natsuyo Aoyama[3], Brad Swanson[3], Jayne Hesley[4], Steve Kattman[3]
[A]Product Management, [B]Applications, [C]R&D, [1]CDI, Madison, WI, United States, [2]Marketing, Molecular Devices, Sunnyvale, CA, United States

Human induced pluripotent stem cell (hiPSC) technology has brought relevant human biology into the laboratory setting. With their human origin, self-renewal, and ease of handling, hiPSCs and hiPSC-terminally differentiated tis-

sue cells overcome many issues associated with more traditional cellular models: including labor-intensive isolation, non-native physiology, variability between donors/suppliers, and insufficient amounts to support large scale investigations and screens. These advantages have enabled the field to expand at an exponential rate whereby practical application and implementation of this technology has furthered basic and applied research into the human condition.

A major challenge in the field of cardiovascular disease research has been the lack of robust and physiologically-relevant cellular systems for drug discovery. Here we demonstrate application of hiPSC-derived cardiomyocytes as an in vitro disease model of cardiac hypertrophy and illustrate how this model may be used in the drug discovery process to identify small molecule therapeutics. Exposure of hiPSC-derived cardiomyocytes to the agonist endothelin-1 (ET 1) leads to classic indicators of the hypertrophic response, including increased cell size, higher sarcomeric organization, and altered expression of components within the fetal gene program (eg. B-type natriuretic peptide, BNP). Based on these key cellular transformations, we developed a series of endpoint readouts including qPCR, flow cytometry, ELISA, and high content imaging (HCI) in 96- and/or 384-well format that provide consistent, reliable, and biologically-relevant results. HCI has the additional advantage of multiplexing biochemical and morphological endpoints, i.e. BNP levels and cell size. In this image-based system, stimulation with ET-1 consistently yielded >20-fold assay windows for detection of BNP with EC50 values = 3.54 ± 2.2 pM.

Cardiac hypertrophy is a multi-factorial disease arising across distinct mechanisms. The presence of multiple overlapping mechanisms may render target-based drug discovery screens less effective in finding therapeutic interventions. Phenotypic screens look for modulation of a physiological endpoint rather than modulation of a single target and thus may show more utility for developing therapeutics against complex diseases such as hypertrophy. To demonstrate the utility of hiPSC-cardiomyocytes and HCI in a phenotypic screen, we profiled a set of tool compounds and found that the hypertrophic response was ameliorated by several unrelated classes of small-molecules, including a calcium channel blocker (verapamil IC50 = 25 nM), an HDAC inhibitor (SAHA, IC50 = 2.8 uM), a p38 MAPK inhibitor (VX-702, IC50 = 8 uM), and cyclosporine A (IC50 = 2.6 uM). The phenotypic screen in this example identified several unique targets that could then be further evaluated as lead molecules within a drug development program.

Human induced pluripotent stem cell-derived cardiomyocytes overcome hurdles presented by traditional cardiac cellular models while HCI analysis enables quantitative high throughput interrogation. By providing a platform for interrogating a disease-state, elucidating underlying mechanisms, and ultimately providing a translatable path forward to ameliorate the pathology, hiPSCs as a model for cardiac hypertrophy exemplifies the power of stem cell technology.

T-3143

NOVEL SMALL MOLECULES PROMOTING CARDIOMOYOCYTE DIFFERENTIATION FROM MURINE AND HUMAN CARDIAC PROGENITOR POPULATIONS

Hiroyuki Fukushima, Jun K. Yamashita
Kyoto Univ Center for IPS Cell Research and Application, Kyoto, Japan

Embryonic
stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are promising cell sources for cardiac regenerative medicine. Previously, we established a 2-dimentional culture-based sequential cardiovascular differentiation system from mouse ESC/iPSCs. This method is amenable to assess differentiation efficiencies at each differentiation stage (undifferentiated ESC/iPSCs, mesoderms, cardiac progenitors, cardiomyocytes). Recently, chemical biological approaches are starting to have an increasingly important role in stem cell biology and regenerative medicine. Some small molecules can efficiently regulate cell fate or modulate cell reprogramming. We reported that an immunosuppressant, cyclosporin-A, showed a novel potent cardiogenic effect specifically acting on Flk-1 positive mesoderm cells to increase cardiomyocytes

by 10 times.
We recently established a high-throughput screening (HTS) based on our ESC cardiovascular differentiation system and chemical approach. Using this HTS of co-culturing mouse ES cells that carry α-myosin heavy chain promoter-driven EGFP gene with OP9 mouse bone marrow-derived stroma cells, we can accurately and efficiently identify chemicals promoting cardiomyocyte differentiation from Flk-1 positive mesoderm. Here we report that we successfully discovered several cardiomyocyte differentiation chemicals (CDCs) from chemical libraries. In a natural chemical library derived from marine invertebrates, we identified a couple of natural chemicals (nCDC). Particularly, nCDC1 showed potent cardiomyocyte induction at nanomolar (nM) level. This active concentration (2.5 nM) was 1000 times lower than cyclosporin-A. And nCDC1 increased cardiomyocyte percentage and cell number that appeared from Flk-1 positive mesoderm cells approximately 20 times more than control.
Next, we examined direct effect of nCDC1 on Flk-1 positive mesodermal cells and ES cell-derived cardiac progenitor cells, $Flk1^{+}/CXCR4^{+}/VE\text{-}cadherin^{-}$ (FCV) cells. nCDC1 potently induced cardiomyocytes from $Flk\text{-}1^{+}$ cells and FCV cells even in the absence with OP9 stromal cells. Next, we examined effects of nCDC1 on human iPS cells. nCDC1 induced cariomyocyte differentiation of KDR^{+} mesoderm derived from human iPS cells. Moreover, nCDC1 promoted cariomyocyte differentiation of a somatic cardiac progenitor population, rat neonate cardiac side population (CSP) cells. These results showed nCDC1 induced cardiomyocytes from various progenitor populations that have potential cardiac differentiaton.
These findings would provide a clue for cardiomyocyte differentiation mechanisms and offer novel cardiac regenerative strategies including cardiac regenerative drugs.

T-3144

THYROID HORMONE PATHWAY MODULATION : EFFECT ON STEM CELL-DERIVED CARDIOMYOCYTE MATURATION

Karine E D Vauchez, Carolina Pinto Ricardo, Gabor Foldes, Maxime Mioulane, Nadire N. Ali, Alexandra E. Kareh, Thusharika Kodagoda, Nicola J L Hellen, Michela Noseda, Michael D. Schneider, Sian E. Harding
NHLI, Imperial College, London, United Kingdom

Introduction : Functional cardiomyocytes can be derived from both embryonic (hESCs) and induced pluripotent (hiPSCs) stem cells. They represent a hope in terms of tissue repair and are being investigated as a cell model system for basic research or drug discovery/toxicology. However, from both hESC and hiPSC, the stem cell-derived cardiomyocyte (SC-CM) phenotype is immature, and resemble foetal or neonatal cardiomyocytes. In the adult heart thyroid hormone (TH) has pleotropic effects on contractility, energy metabolism and mitochondrial remodelling. During devlopment, Deiodinase 3 (D3) metabolises TH into inactive products to protect the foetus against high maternal T3 levels. Down regulation of D3 at appropriate times during development allows T3 action on physiological growth and maturation of the heart. The aim of this study is to drive SC-CM to a more mature state by modulation of TH pathway.
Methods and Results : hiPSC-CM, (Cellular Dynamics) were treated with 3nM or 30nM Triiodothyronine (T3) for up to 2 weeks. Gene expression of TH target genes that are key markers of cardiac maturation or oxidative metabolism were assessed by qPCR.
The expression of beta 1 Adrenergic Receptor was increased in T3 treated cells and remained stable for up to 2 weeks ($p<0.05$). The alpha/beta myosin heavy chain ratio was increased in correlatation with T3 concentrations at both 1 and 2 weeks ($p<0.001$). ATP synthase beta subunit (involved in the electron transport chain) expression level was increased after 2 weeks of treatment with 30nM of T3 ($p<0.05$). No significant change in gene expression was observed for Sarcoplasmic Reticulum Ca^{2+}-ATPase, pyruvate dehydrogenase kinase 4, cytochrome c and cytochrome b. Immunostaining for troponin T and TMRM (mitochondrial membrane potential, dye) was used to measure cell size and mitochondrial density and network organisation respetivly. Nuclei were stained with Hoechst. Plates were scanned on ArrayScan™ VTI automated microscopy and image analysis system (Cellomics). T3 did not increase hiPSC-CM size. No modification of the mitochondrial density or network organisation was observed on treateted cells with T3.
D3 gene expression was compared by qPCR in hiPSC-CM, hESC (undifferentated H7 cell line) and hESC-CM, generated 1) via embryoid body (EB) formation, or 2) from dense cell monolayers treated with Activin A and bone morphogenetic protein 4 (Activin A/BMP4). hESC-CM were maintained in culture for up to 6 months. At 1 month post-differentation, D3 expression in the hiPSC-CM was very low in comparison to hESC-CM. Normalized by hESC, D3 expression levels of hiPSC-CM, hESC-CM via Activin A/BMP4 and hESC-CM via EB were 0.02 +/- 0.01, 9.38 +/- 0.24 and 2.06 +/- 0.7 respectively ($p<0.001$). In comparison to hESC, a peak in D3 expression was seen at 1 and 2 months post-differentiation of hESC-CM (9.38 +/- 0.24 and at 8.53 +/- 2.35 time increase respectively ; $p<0.001$) and then decreased.
Conclusion : T3 treatment of hiPSC-CM induced modulation of some T3 specific target genes and/or function including key markers of cardiac maturation and oxidative metabolism at different time points. Low D3 expression level in hiPS-CM in comparison to hESC-CM indicated a difference between stem cell sources. Moreover D3 could be an intrinsic control mechanism of hESC-CM maturation. For this reason simultaneous application of T3 and down-regulation of D3 could be an effective strategy to drive the maturation of hESC-CM.

T-3145

HESC-DERIVED CARDIAC MICRO-TISSUE PARTICLES FOR HEART REGENERATION

Kareen L. Kreutziger[1], Kaytlyn A. Beres[2], Sarah Dupras[1], Xiulan Yang[1], Veronica Muskheli[1], Charles E. Murry[1]
[A]Pathology, [B]Bioengineering, [1]University of Washington, Seattle, WA, United States

Coronary heart disease is now the leading cause of death worldwide, with no curative therapies except total heart transplant. Novel therapies are needed that will correct the contractile deficiencies of the heart after myocardial infarction, namely by cellular remuscularization that is electrically and mechanically integrated with damaged host tissue. Toward this goal, we have designed scaffold-free engineered cardiac “micro-tissue particles” using human embryonic stem cell (hESC)-derived cardiomyocytes for minimally-invasive injection into the heart wall as a regenerative medicine therapy. Cardiomyocyte differentiation is directed using activin A and BMP4 in high density monolayers and yields >50% cTnT-positive cardiomyocytes by flow cytometry analysis. Micro-tissue particles are formed overnight in PDMS microwells so that 1000 and 2000 cells per particle yield 184 ± 2 μm and 243 ± 2 μm diameter spherical cell aggregates, respectively. A myocardial infarction is induced in athymic rats by 60 minutes of ischemia, followed by reperfusion. Four days after injury, 10 million hESC-cardiomyocytes are injected

intramyocardially as micro-tissue particles or a single cell suspension (control). At 2 and 4 weeks, MTP injections were equivalent to cell injections based on graft size by histology and global heart function by echocardiography. However, MTP grafts demonstrated superior graft-host electrical coupling by ex vivo imaging using a fluorescent, genetically encoded calcium indicator GCaMP3 that showed calcium waves synchronous with the host ECG. All MTP grafts were able to follow electrical stimulation up to 6 Hz (360 beats per minute), whereas no cell grafts were detected by ex vivo imaging. While preliminary, these results demonstrate that implantation of engineered cardiac tissue intramyocardially using micro-tissue particles promotes electrical integration of the graft with the host, which may reduce arrhythmogenesis and is a prerequisite for contractile integration. In conclusion, cardiac micro-tissue particle therapy for heart regeneration utilizes tissue engineering to maintain cell-cell and cell-matrix interactions while delivering the engineered tissue by minimally invasive needle injection into the wall of the heart and is a promising approach for remuscularizing the heart with hESC-cardiomyocytes after myocardial infarction.

T-3146

CARDIOXPRESS: STEM CELL DERIVED CARDIOMYOCYTES APPLIED FOR A HIGH THROUGHPUT ELECTROPHYSIOLOGY ASSAYIN CARDIAC SAFETY PHARMACOLOGY

Thomas Meyer[1], Stefan Braam[2], Christophe Bleunven[3], Philippe Zitoun[3]

[1]Multi Channel Systems MCS GmbH, Reutlingen, Germany, [2]Pluriomics, Leiden, Netherlands, [3]NotocordSystems SASSA, Croissy sur Seine, France

The prolongation of the QT interval in the ECG has been one of the most prevalent reasons for withdrawal of novel drug candidates in late preclinical drug development and clinical trials. Industry applied a wide battery of assays to evaluate the arrhythmogenic potential and the probability of QT prolongation in preclinical safety pharmacology. However due to the lack of adult human ventricular cells or tissue mostly animal models are used. Those are lacking full transferability to humans. Here we present an assay based on human stem cell (ES and iPS) based cardiomyocytes. While this concept has been described and evaluated previously, we combine it with high throughput 96 well plate based assay platform as well as 21CFRp11 compliant software modules for acquisition and analysis.
Here we present the cell lines used and evaluated for this assay and demonstrate recordings of those cells using Microelectrode array technology. We also present exemplary drug effects on the cells using standard tool compounds.
The concept of the hardware is based on a 96 well sensing and recording platform incorporating automated liquid handling for compound application and sample preparation. This enables unsupervised operation of the robotic platform.
Data acquisition and analysis will be available in a compliant software suite supporting experimental flow control as well as analysis and automatic report generation.
Matching cells, robotics and software create a HTS compatible versatile high content assay platform vastly improving the convenience and throughput of stem cell electrophysiology in a safety pharmacology environment.

T-3147

INVESTIGATING THE GENETIC CAUSATION OF HYPOPLASTIC LEFT HEART USING INDUCED PLURIPOTENT STEM CELLS (IPSC)

Alexis Bosman[1], Gillian Blue[2], David Winlaw[2], Richard P. Harvey[1]

[1]Victor Chang Cardiac Research Institute, Darlinghurst, Australia, [2]The Children's Hospital at Westmead, Heart Centre for Children, Westmead, Australia

Hypoplastic left heart (HLH) is a congenital heart defect (CHD) characterized by varying degrees of underdevelopment of the left ventricle and other left-sided heart structures. HLH is the most severe form of CHD and although it is relatively uncommon (~2-3% of all CHD), without treatment it would account for 25-40% of all neonatal cardiac deaths. Kindred analysis suggests that HLH has a large genetic component with complex inheritance patterns suggestive of a multifactorial cause. While many attribute HLH to altered hemodynamics, we hypothesize that HLH

occurs at the intersection of altered hemodynamics due to inflow and/or outflow constriction (environment) and a fundamental genetic deficit within ventricular myocardium during development. To address our hypothesis, we have created iPSC lines from HLH patients as well as both parents, thus providing controls that are as genetically closely matched to the patient as possible. Using these iPSCs and cardiac differentiation protocols, we are able to recapitulate early cardiac development in vitro, therefore allowing us to examine perturbations in the cardiac gene network from the earliest time points. To date, iPSC lines (3 clones each) from two HLH probands and their parents have been generated and characterized. Preliminary data using an embryoid body differentiation protocol show differences in the number of beating cardiomyocytes and expression of cardiac and smooth muscle genes at day 21 of differentiation.

T-3148

TRANSPLANTATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-ENGINEERED TISSUE SHEETS WITH DEFINED CARDIOVASCULAR CELL POPULATIONS FOR INFARCTED RAT HEARTS

Hidetoshi Masumoto[1], Akira Marui[2], Tadashi Ikeda[2], Tatsuya Shimizu[3], Teruo Okano[3], Ryuzo Sakata[2], Jun K. Yamashita[1]

[1]Center for iPS Cell Research and Application (CiRA) Kyoto Univ, Kyoto, Japan, [2]Department of Cardiovascular Surgery, Kyoto University Graduate School of Medicine, Kyoto, Japan, [3]Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan

BACKGROUNDS: To realize cardiac regeneration using human induced pluripotent stem cells (hiPSCs), efficient differentiation from hiPSCs to defined cardiac populations (cardiomyocytes [CMs]/ endothelial cells [ECs]/ vascular mural cells [MCs]), and transplantation technique for fair engraftment are required. Recently, we reported that mouse ES cell-derived cardiac cell sheet transplantation to rat myocardial infarction (MI) model ameliorated cardiac dysfunction (Masumoto, Stem Cells 2012). Here we tried to extend this strategy to hiPSCs. METHODS & RESULTS: We have reported an efficient CM differentiation protocol from hiPSCs based on a monolayer culture (PLoS One, 2011), in which cardiac troponin-T (cTnT)-positive CMs robustly appeared (50-80%). In this study, we further modified the protocol to induce vascular cells (ECs/MCs) together with CMs by vascular endothelial cell growth factor supplementation, resulted in proportional differentiation of cTnT+-CMs (60.6±12.4% of total cells), VE-cadherin+-ECs (7.7±4.7%) and PDGFRb+-MCs (17.7±11.2%) at differentiation day 15 (n=15). Then, these cells were transferred onto temperature-responsive culture dishes (UpCell; CellSeed, Tokyo, Japan) to form cardiac cell sheets with defined cardiac populations. After 4 days of culture, we successfully collected self-pulsating cardiac cell sheets with 7.6×105±2.6 (n=15) of cells containing CMs (48.8±14.6% of total cells), ECs (3.9±3.5%), and MCs (23.3±17.2%). Multichannel extracellular potential analysis revealed that the cardiac tissue sheets hold unidirectional and regular electrical propagation, with no ectopic foci (MED 64 system). A regular calcium transient was observed throughout the sheet along with spontaneous beating. The cell sheets were transplanted to a MI athymic rat heart one-week after MI. In transplantation group, echocardiogram showed a significant improvement of systolic function of left ventricle (fractional shortening: 22.5±4.8 vs 36.2±7.8%, $p<0.001$, n=24) and a decrease in akinetic length (20.6±9.3 vs 2.1±7.0%, $p<0.001$, n=24) (pre-treatment vs 4weeks after transplantation). We confirmed a prominent accumulation of vWF-positive endogenous ECs around the graft within three days after transplantation, indicating angiogenic effects of the sheet. Engrafted human cells mainly consisted of CMs were still observed in over one-third of transplanted rats four weeks after transplantation (4 in 11 rats; 36.4 %, engrafted area / infarcted area: 28.0±23.4 %; 3.5-59.8%). CONCLUTIONS: Transplantation of cell sheets with hiPSC-derived defined cardiac populations ameliorates cardiac dysfunction after MI. Thus, we developed a valuable technological basis for hiPSC-based cardiac cell therapy.

T-3151

CHARACTERISATION OF CARDIOMYOCYTES DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM (IPS) CELLS

Franziska Sendfeld[1], Claire N. Medine[1], Olga Tura Ceide[1], Fabiana S. Scornik[2], Guillermo J. Pérez[2], Ramon Brugada[2], Ian Wilmut[1], Nicholas L. Mills[1]

[1]University of Edinburgh, Edinburgh, United Kingdom, [2]Cardiovascular Genetics Center, UdG-IdIBGi, University of Girona, Girona, Spain

Objective: The ability to derive cardiomyocytes from human induced pluripotent stem (iPS) cells provides a novel approach to study inherited cardiac channelopathies with advantages over existing model systems. Dermal fibroblasts from patients with a defined cardiac phenotype or genetic mutation can be reprogrammed to iPS cells and differentiated into cardiomyocytes with the same genetic background. Whilst this technique has enormous potential to model inherited channelopathies, such as Brugada Syndrome, the derived cells have not been fully characterised and compared to foetal and adult cardiomyocytes. Knowing the maturity of iPS cell derived cardiomyocytes will be critical if assessments of cellular function are to provide insight into disease processes that emerge in the adult.
Methods: Human embryonic stem (ES) cells and iPS cells obtained from healthy controls were differentiated into cardiomyocytes using an unguided differentiation protocol. Embryoid bodies from undifferentiated ES and iPS cells were cultured in suspension for 4 days and then plated onto 0.1% gelatin. Spontaneously contracting areas were disaggregated and single cardiomyocytes were characterised using immunocytochemistry, RT-PCR, electrophysiology and Ca2+ imaging. Cells were stained for proteins of the contractile apparatus like Troponin T, Troponin I and α-actinin. Ca2+ release into the cytoplasm was detected by fluo-4 which fluoresces upon binding Ca2+. Fluorescence intensity was traced before and after Tetrodotoxin (TTX) treatment. Sodium channel activity was measured in a low sodium buffer using perforated patch-clamping. Derived cardiac cells were then compared to primary cardiomyocytes isolated from human foetal and adult cardiac tissue.
Results: ES and iPS derived cardiomyocytes express a wide range of cardiac markers such as GATA-4, α-actinin and troponin I. They express Tetrodotoxin resistant voltage activated sodium channels that inactivate and recover from inactivation and exhibit action potential triggered Ca2+-induced-Ca2+-release. Spontaneous contraction was observed in derived cardiomyocytes and immature human foetal cardiomyocytes.
Conclusion: Although iPS cells give rise to a mixture of immature and more mature cardiomyocytes, they all express typical cardiac proteins and have functional cardiac sodium channels. This differentiation system may be utilised to investigate patients with and without known genetic mutations to provide a better understanding of the pathophysiology of inherited cardiac channelopathies.

T-3152

ROLE OF MICRORNAS IN EMBRYONIC STEM CELL DERIVED CARDIOMYOCYTE MATURATION

Kavitha T. Kuppusamy[1], Savannah Cook[2], Anup Madan[4], James Fugate[3], Benjamin Van Biber[3], Lil Phabon[3], Carol Ware[2], Michael Laflamme[3], Charles E. Murry[3], Hannele Ruohola-Baker[1]

[A]Biochemistry, [B]Comparative Medicine, [C]Pathology, [1]University of Washington, Seattle, WA, United States, [2]Covance Incorporation, Seattle, WA, United States

Heart failure is a burgeoning public health problem that results principally from a deficiency of cardiomyocytes. The ability to derive cardiomyocytes from stem cells offers the potential of addressing this root cause, either through transplantation therapies or manipulation of endogenous cardiac cells. However, embryonic stem cell derived cardiomyocytes have only fetal stage maturity making them poor model of adult cardiomyocytes and poorly functioning in *in vivo* applications. If we hope to use human embryonic stem cell (hESC) derived cardiomyocyte to model genetic diseases with adult onset such as cardiomyopathies, screen drugs, model adult signaling and physiology or repair the damaged heart, the maturation of stem cell-derived cardiomyocytes need to be accelerated. Our preliminary data suggest that microRNAs control maturation of hESC-derived cardiomyocytes. In this project, we will further test this hypothesis and attempt to accelerate cardiomyocyte maturation by manipulating key miRNA function. Large scale miRNA sequencing and candidate approaches have revealed interesting families of miRNAs to be highly expressed in mature cardiomyocytes. Functional analysis through gain and loss of function strategies and tar-

get analysis will provide further insights into the mechanistic action of these miRNAs. We believe that this work will be highly significant for basic and applied research and regenerative medicine since it will enable the production of mature cardiomyocytes from hESCs/hiPSCs and potentially from endogenous cardiac stem cells *in vivo*, thereby increasing the value of stem cell cardiogenesis for medical applications.

T-3153

SINGLE-CELL PCR ANALYSIS OF C-KIT+ CD45- CARDIAC STEM CELLS REVEAL HETEROGENEITY IN REGARD OF CARDIAC AND ENDOTHELIAL COMMITMENT

Joakim Sandstedt[1], Marianne Jonsson[1], Göran Dellgren[2], Anders Lindahl[1], Anders Jeppsson[2], Julia Asp[1]

[1]Department of Clinical Chemistry and Transfusion Medicine, Institute of Biomedicin, the Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [2]Department of Molecular and Clinical Medicine, Institute of Medicine the Sahlgrenska Academy, University of Gothenburg and Department of Cardiothoracic Surgery, Gothenburg, Sweden

Introduction: C-kit+ cells within the heart has previously been described as multipotent stem cells capable of giving rise to cardiac, endothelial and smooth muscle cells, however most studies of differentiation have been conducted in animal models. In a previous study on human cardiac C-kit+ CD45- cells, we were not able to detect any evidence of cardiac specification but rather endothelial commitment within the total C-kit+ CD45- population. We have hypothesized that there is a small fraction of C-kit+ CD45- cells that have a cardiac specification.
Purpose: To investigate heterogeneity within the human cardiac C-kit+ CD45- population in regard of cardiac, endothelial and stem cell properties using single-cell PCR.
Methods: Right and left atrial biopsies were obtained after informed consent from patients undergoing cardiac surgery. The tissue was dissociated and the cardiomyocyte depleted cell suspension was subjected to multi color flow cytometry. C-kit+ CD45- cells were sorted as single cells to 96 well plates, then analyzed by single-cell PCR for expression of cardiac, endothelial and stem cell genes.
Results: Only right atrial biopsies contained a clearly distinguishable C-kit+ CD45- population. A total of 471 single C-kit+ CD45- cells from five biopsies were sorted. The cells were screened for *C-KIT* gene expression to confirm C-kit+ identity. An average of 75% of the sorted cells had a clear expression of *C-KIT* also at the gene level. These cells were then analyzed for expression of cardiac gene *NKX2.5* and endothelial gene *VWF* to evaluate cardiac and endothelial commitment, respectively. A minor portion of the cells (1.1%) expressed *NKX2.5* while the majority of cells (81%) expressed *VWF*. The remaining *VWF* negative cells were further analyzed for a wider panel of genes. Since *NKX2.5+* cells were of special interest, all *NKX2.5+* cells regardless of *VWF* expression were also included. A part of the analyzed cells were found to express other endothelial genes (*FLK-1, CD31*) instead of *VWF* while another part expressed late cardiac genes (*TNNT2, ACTC1*). Embryonic stem cell gene *OCT4* was found to be expressed in a minor portion of the selected cells.
Conclusions: C-kit+ CD45- cells were heterogeneous in regard of cardiac and endothelial differentiation. While most cells expressed markers for endothelial genes, a minor portion was found to express both early and late cardiac genes. This indicates that there is a sub-population committed to cardiac differentiation within the C-kit+ CD45- population.

T-3154

HEMOGENIC ENDOCARDIUM CONTRIBUTES TO TRANSIENT DEFINITIVE HEMATOPOIESIS

Haruko Nakano[1], Xiaoqian Liu[1], Armin Arshi[1], Yasuhiro Nakashima[1], Ben van Handel[1], Rajkumar Sasidharan[1], Andrew W. Harmon[1], Jae-Ho Shin[1], Robert J. Schwartz[2], Simon J. Conway[3], Richard P. Harvey[4], Mohammad Pashmforoush[5], Hanna K. A. Mikkola[1], Atsushi Nakano[1]

[1]MCDB, UCLA, Los Angeles, CA, United States, [2]Biology and Biochemistry, University of Houston, Houston, TX, United States, [3]the Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis,

IN, United States, [4]Developmental Biology Division, University of New South Wales, New South Wales, CA, United States, [5]USC, Los Angeles, CA, United States

Hematopoietic cells arise from spatiotemporally restricted domains in the developing embryo. Although studies of non-mammalian animal and in vitro embryonic stem cell models suggest a close relationship among cardiac, endocardial, and hematopoietic lineages, it remains unknown whether the mammalian heart tube serves as a hemogenic organ akin to the dorsal aorta. Here, we examine the hemogenic activity of the developing endocardium. Mouse heart explants generate myeloid and erythroid colonies in the absence of circulation. Hemogenic activity arises from a subset of endocardial cells in the outflow cushion and atria earlier than in the aorta-gonad-mesonephros region, and is transient and definitive in nature. Interestingly, key cardiac transcription factors, Nkx2-5 and Isl1, are expressed in and required for the hemogenic population of the endocardium. Together, these data suggest that a subset of endocardial/endothelial cells expressing cardiac markers serve as a de novo source for transient definitive hematopoietic progenitors.

T-3155

MOLECULAR INSIGHTS ON THE FUNCTIONAL ROLE OF TESTOSTERONE MEDIATED STEM CELL CARDIOMYOGENESIS

Ashraf S. Madhoun[1], Anastassia Voronova[2], Abeer Zakariyah[2], Tammy Ryan[2], Ilona S. Skerjanc[2]
[1]Dasman Diabetes Institute, Dasman, Kuwait, [2]Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada

Background - Androgens play an important role in heart development. Studies have shown that mouse embryonic stem (mES) cells express the androgen receptor (AR) in a differentiation-dependent fashion and secrete low levels of testosterone. Further, during embryonic body induced differentiation, testosterone enhanced the formation of beating cardiomyocytes via the classical AR-mediated genomic pathway. In addition, AR-deficient mice exhibited reduced heart-to-body weight ratios, impaired ventricular function and the development of cardiac abnormalities in the adult. Together, these findings indicate that testosterone has a critical role in cardiac muscle development, but the stages and mechanisms of testosterone action require further elucidation.
Aim - The aim of this study is to elucidate the molecular mechanisms regulating testosterone-enhanced cardiomyogenesis in stem cells. This will provide deeper insight into the functional role of androgen pathway in the differentiation of stem cells into cardiac muscle.
Methods and Results - Real time PCR analysis showed that testosterone upregulated cardiomyogenic transcription factors, including GATA4, MEF2C, and Nkx2.5, muscle structural proteins, and the pacemaker ion channel HCN4 in a dose-dependent manner, in mouse embryonic stem (mES) cells and P19 embryonal carcinoma cells. In concentration dependent fashion, treatment with anti-androgenic compounds reversed the action of testosterone and inhibited cardiomyogenesis, indicating a requirement for AR. These observations were further confirmed in AR knockdown studies using stable cells lines expressing AR-targeted short haipin RNA (shRNA). Chromatin immunoprecipitation (ChIP) studies showed enhanced and selective recruitment of AR to the cardiac muscle regulatory regions of *MEF2C* and *HCN4*, leading to a targeted increase in histone acetylation at the cardiac enhancers. The cardiac specific regulatory regions were further conformed using bioinformatics utilizing ENCODE/LICR database analysis. Finally, testosterone-enhanced cardiomyogenesis required Ca2+ influx and led to the upregulation of the L-type calcium channel (DHPR) CACNA1S, implying a possible implication of non-genomic AR-pathway.
Conclusion - Testosterone enhanced cardiomyogenesis by upregulating cardiomyogenic transcription factors, at least in part by recruiting the AR to the cardiac regulatory regions of *MEF2C*. Furthermore, testosterone recruits the AR to the regulatory regions of *HCN4* and upregulated its expression. These results provide a detailed molecular analysis into the function of testosterone in stem cells and provide molecular insight into the negative consequences of testosterone abuse in humans, which include cardiac hypertrophy and arrhythmias.

T-3156

FUNCTIONAL COUPLING OF STEM-CELL DERIVED AND PRIMARY CARDIAC MYOCYTES

Yvonne Aratyn-Schaus, Francesco S. Pasqualini, Megan L. McCain, Sean P. Sheehy, Kevin K. Parker
Harvard University, Cambridge, MA, United States

A major challenge of clinical trials investigating cardiac stem cell-based therapy is to establish efficacy in the treatment of ventricular dysfunction post myocardial infarction. While these trials have shown a positive safety profile, pre-clinical data indicate variable electrical-coupling and limited engraftment of ESC-derived cardiomyocytes to the native myocardium. These findings raise the question of whether they can successfully transmit force across the cell-cell boundary. Here, we describe an *in vitro* assay to study mechanical coupling between primary cells and ESC-derived cardiomyocytes. We hypothesize that ESC-derived cardiomyocytes form immature intercellular junctions with primary cardiomyocytes that limit force transmission across the cell-cell junction. An *in vitro* co-culture assay using micro-contact printed cell pairs composed of ESC-derived and primary cardiac myocytes was designed to test this hypothesis. High-resolution Traction Force Microscopy was used to quantify force-distribution across cell-cell and cell-substrate interfaces. We found that ESC-derived cardiac myocytes form mechanical linkages with primary cardiomocytes that support synchronous contraction, but incomplete development of adherens junctions restricted force transmission between cells. To compensate, intermediate forces were exerted on the substrate, near cell-cell junctions. These results suggest that engraftment and adhesion are necessary but not sufficient conditions for successful cardiac stem cell therapy, and that force-dissipation between engrafted cells and native cardiomyocytes may explain the incomplete repair of ejection fraction in clinical studies.

Eye or Retinal Cells

T-3161

HIGH FIDELITY EPISOMAL CB-IPSC GENERATE VASCULAR PROGENITORS WITH REDUCED SOMATIC MEMORY AND AUGMENTED CAPACITY FOR REGENERATING THE ISCHEMIC RETINA

Tea Soon Park[1], Imran Bhutto[2], Ludovic Zimmerlin[1], Jeffrey Huo[1], Pratik Nagaria[4], Diana Miller[5], Rufaihah Abdul Jalil[6], Connie C. Talbot[3], Ricardo Feldman[5], Feyruz Rassool[5], John Cooke[6], Gerard Lutty[2], Elias Zambidis[1]
[A]Institute for Cell Engineering, [B]Wilmer Ophthalmological Institute, [C]Institute for Basic Biomedical Science, [1]Johns Hopkins School of Medicine, Baltimore, MD, United States, [A]Department of Radiation Oncology, [B]Department of Microbiology/Immunology, [2]University of Maryland, Baltimore, MD, United States, [3]Department of Cardiovascular Medicine, Stanford University, San Francisco, CA, United States

Human induced pluripotent stem cells (hiPSC) offer great potential as an alternative source of vascular progenitors (VP) for treatment of ischemic disorders. We recently reported an efficient human embryoid body (hEB)-based method to derive hemato-vascular progenitors from human pluripotent stem cells (hPSC). In this study, we characterized VP generated from non-integrated cord blood (CB)-hiPSC, fibroblast-hiPSC and human embryonic stem cells (hESC), and assessed their in vivo homing and engraftment potential in a murine retinal ischemia-reperfusion (I/R) model.
VP derivation was achieved via a defined multistep hEB differentiation that utilized mesodermal induction followed by vascular specialization in growth factor-driven monolayer cultures. VP populations were purified by FACS based on expression of the endothelial/pericytic markers CD31 and CD146, characterized in angiogenic conditions by flow cytometry and q-RT-PCR, and tested for vasculogenic potential in vitro (Dil-acetylated-LDL uptake, Matrigel tube assay) and in vivo (Matrigel plug assay). We observed that CB-iPSC (23.2±2.7%, n=17) derived via a novel stromal-primed (sp) reprogramming method generated significantly higher percentages of CD31+CD146+ VP compared to fibroblast-iPSC (13.6±1.8%, n=11), and at levels comparable to hESC (19.9±1.7%, n=13). CB-iPSC-VP also retained immature angiogenic markers (e.g., KDR, CD90, Tie1, Tie 2) at higher levels than fibroblast-iPSC after expansion. Senescence and apoptosis assays demonstrated that sp-CB-iPSC-derived VP were significantly less senescent than fibroblast-iPSC-VP and displayed greater DNA repair capacities. We employed bioinformatic ANOVA methods from whole genome transcriptomic analysis of undifferentiated hPSC and their CD31+CD146+ VP derivat-

ives to reveal that sp-CB-iPSC and their VP derivatives possessed significantly less somatic memory-associated and aberrant gene expression. To determine the functional performance of CD31+CD146+ VP cells derived from hESC (n=26), fibroblast-iPSC (n=5) and CB-iPSC (n=22), we injected them into an adult murine ocular ischemia-reperfusion injury model. Sp-CB-iPSC-derived VP engrafted up to 45 days via local (vitreous body) or systemic (orbital sinus and tail vein injections) routes. In contrast fibroblast-iPSC-derived VP displayed only limited short-term engraftment potential (3-7 days). Both hESC- and CB-iPSC-VP engrafted preferentially into damaged capillaries and veins rather than arteries, at abluminal and luminal locations, forming elongated chimeric blood vessels, as revealed by the detection of multiple human vascular cells in the mouse retinal vasculature. Collectively, these data suggested that augmented generation and higher functional in vivo performance of sp-CB-iPSC-VP was due to their high fidelity pluripotency that contained fewer reprogramming errors. Unlike VP derived from other sources, sp-CB-iPSC-VP were highly responsive to injury signals and efficiently incorporated into damaged retinal vasculature.
In summary, the use of CB is attractive for regenerative vascular therapies since this somatic source carries relatively few somatic mutations, and could be used to create an HLA-defined stem cell bank via worldwide networks of existing blood bank repositories. Thus, these studies provide a valuable preclinical model for evaluating the potential for generating transplantable VP for treatment of ischemic retinopathy.

T-3162

MONITORING STEM CELL TRANSPLANTS IN OCULAR DISEASE

Roxanne H. Croze
MCDB, UC Santa Barbara, Santa Barbara, CA, United States

Age related Macular Degeneration (AMD) is a progressive disease leading to loss of fine acuity vision. More than 200,000 people are diagnosed per year, making AMD the leading cause of blindness in the elderly in the developing world.
Current research for development of an AMD therapeutic focuses on using human embryonic stem cells (hESC). hESCs spontaneously differentiate into RPE after the removal of basic fibroblastic growth factor and can be enriched to create a homogenous population of cells. Clinical trials are already underway using bolus injections of hESC-RPE. In addition, a second transplantation approach is being studied that involves culturing hESC-RPE on a synthetic substrate and transplanting the monolayer into the eye. To date, no studies have been done to compare these two approaches, and methods are lacking to monitor cells after transplantation.
To address this issue, molecular tools need to be created to examine parameters of cell survival, function, proliferation, migration, and population homogeneity after transplantation in real time. We are developing lentiviral vectors where specific gene promoters drive expression of different fluorescent genes to visualize cell behavior.
Current work has created numerous reporters, including vectors where EF1α, Ki67, Best1, and RPE65 promoters respectively drive eGFP expression, and an Otx2 promoter that drives mCherry expression. EF1α, a constitutive marker, will track migration of transplanted cells. The Ki67 reporter will monitor mitotic activity. Reporters for RPE specific genes, Best1, RPE65, Otx2 track RPE cell identity. In addition, a caspase 3 FRET based reporter monitors induction of apoptosis. Reporter constructs have been transduced into hESC-RPE and appropriate control cell lines, and expression was assessed via fluorescence microscopy. Expression of RPE-specific promoters was observed in hESC-RPE cells, but not in negative controls. Expression of Ki67 and caspase-3 was observed in dividing cells and apoptotic cells, respectively.
hESC-RPE cells containing reporters will be grown on substrates and transplanted into the RCS rat, a model of retinal dysfunction. Real time imaging post-transplantation will reveal reporter expression in vivo. Imaging results will identify key behaviors of transplanted hESC-RPE to guide development of cellular therapies for those suffering from the advanced form of Dry AMD.

T-3163

ENHANCEMENT OF MÜLLER GLIA REGENERATIVE FEATURES: HOW TO IMPLEMENT PROLIFERATION WITHOUT IMPARING DIFFERENTIATION INTO PHOTORECEPTORS.

Serena G. Giannelli, Vania Broccoli
Neuroscience, San Raffaele Scientific Institute, Milano, Italy

The capability of Müller Glia cells (MGCs) to regenerate to some extent the neurons and more importantly the photoreceptor of the retina has already been widely demonstrated in all vertebrates classes and more recently even in humans (Giannelli SG et al., 2011). Nonetheless these achievements remain speculative and yet far from a translational application given the impossibility to expand MGC in a large scale.
Given these premises we set to establish a procedure to overcome MGC proliferative limit by immortalizing them in a transient fashion, thus preserving their ability to differentiate into photoreceptors and to generate an efficient source of transplantable cells.
In order to achieve this goal two approaches were tested the first one consisting in transient over-expressing genes known to affect MGC or retinal progenitors proliferation. The expression of these genes, among which: Pax6, Otx2, Sox2, Rx but also Lin28 and Ascl1, was achieved using viral inducible vectors under the control of tetracycline promoter and co-infecting with its transactivator (rtTA). Most of these genes were able to increment (by 2 0 3 fold) proliferation and retard senescence of our cell culture but none was able to induce cell immortalization, not even transiently. None of these treatments altered the karyotype of our cells.
Given the importance of MGC transient immortalization we devise second approach to fulfill it: the down-regulation in MGCs of tumor suppressor genes. These genes promote quiescence and prevent cells from proliferating, thus their inactivation would prompt cells to re-enter cell cycle. We focused our interest on 3 tumor suppressor genes: Rb, Arf and Ink4a, since their knock-down was already demonstrated to convert differentiated myofibers into myocytes, capable of dividing (Pajacini et al., 2010). We coupled shRNA technology with inducible vector technology in order to transiently knock-down these transcripts. Indeed MGCs infected with these vectors changed in their morphology and acquired a faster rate of proliferation. Thus far we were able to passage these lines for at least 20 passages, as compared to mock calls that underwent senescence upon 4-5 passages.
MGCs immortalization therefore is an efficacious tool to transiently amplify MGC pool and thus enable us to exploit its potential in regenerating the retina.

T-3164

DIFFERENTIATION OF NEURAL CREST-DERIVED CORNEAL STROMA STEM CELLS INTO CORNEAL KERATOCYTES IN VIVO.

Emi Inagaki, Satoru Yoshida, Shin Hatou, Hideyuki Miyashita, Tetsuya Kawakita, Kazuo Tsubota, Shigeto Shimmura
Ophthalmology, Keio University, Tokyo, Japan

Purpose:
Currently, corneal transplantation is the only method of restoring corneal clarity and function to scarred corneas. However, the procedure has limitations such as graft failure, immunological rejection and shortage of donors. Recently, new approaches to solve this problem include corneal tissue engineering and stem cell therapy. We previously reported that stem cells isolated from the adult corneal stroma, (cornea-derived precursors(COPs)) show characteristics of multipotent neural crest-derived stem cells. In this study, we report induction of corneal keratocyte from COPs *in vivo*.
Methods& Results:
Cornea-derived precursors (COPs) cells were isolated from C57BL6J mouse corneas. After GFP labeling by Lentivirus vector, COPs were injected into the stroma of mouse corneas (10^4 cells /cornea.) Transplanted COPs survived in the mouse cornea stroma for more than one month. In addition, these cells assumed a keratocyte dendritic morphology. However, expression of keratocyte specific keratan sulfated keratocan was not detected by immunohistochemistry. We then made a decellularized model of the mouse cornea by trans-corneal cryopexy using a stainless probe (diameter 2 mm) cooled with liquid nitrogen that was applied to the corneal surface for 20 seconds.
In this model, approximately half of the resident corneal stromal cells died on the next day. COPs were injected into decellularized B6 mouse corneas on the following day after cryopexy. Transplanted COPs survived in the mouse

cornea stroma for more than one month. These cells assumed a keratocyte dendritic morphology. Moreover, expression of keratocyte specific keratocan was detected by immunohistochemistry.
Conclusion:
Intrastromal transplantation of Cornea-derived precursors (COPs) is a promising cell source for the treatment of corneal stromal disease.

T-3165

HNPPFV CELLS OVEREXPRESSING ECTOPIC IGF-1 ENHANCE RETINAL GANGLION CELL SURVIVAL AND NEURITE OUTGROWTH

Jie Ma, Chenying Guo, Kameran Lashkari
Schepens Eye Research Institute, Massachusetts Eye & Ear Infirmary, Department of Ophthalmology, Harvard Medical School, Boston, 02114 MA, Boston, MA, United States

hNPPFV Cells Overexpressing Ectopic IGF-1 Enhance Retinal Ganglion Cell Survival and Neurite Outgrowth
Jie Ma, Chenying Guo, Kameran Lashkari
Schepens Eye Research Institute, Massachusetts Eye & Ear Infirmary, Department of Ophthalmology, Harvard Medical School, Boston, 02114 MA
Previous studies have shown that IGF-1 acts as an indispensable factor on proliferation and survival of neuron *in vitro* and *in vivo*. Our preliminary observation also indicated that IGF-1-enriched culture medium enhanced the survival and neurite outgrowth of RGCs. In parallel, we have recently shown that neural progenitor cells isolated from human persistent fetal vasculature (hNPPFV cells) can integrate into the RGC layer after transplantation into the mouse vitreous. Immunocytostaining indicated that these cells integrated into the RGC and nerve fiber layers and specifically differentiated into RGC-like cells. Based on these observations, we assessed whether hNPPFV cells could be good candidate cells for locally delivery of neuroprotective factors to the inner retina and to confer clinically relevant global neuroprotection against RGC loss. We chose IGF-1 as a model and cloned mouse IGF-1 cDNA into pJ603-neo vector expressing a red fluorescence reporter gene (tdTomato) to generate fluorescent fusion proteins and tranfected these vectors into hNPPFV cells. We compared the effects of secreted IGF-1tdTomato fusion protein or tdTomato protein alone on apoptosis and axonal growth responses of RGCs in a co-culture system. We also studied the effects of two IGF-1 analogs (H-1356 and NBI-31772), as well as an IGF-1 receptor blocking antibody. In co-culture condition, hNPPFV$^{igf\text{-}1\text{-}tdTomato}$ cells were shown to secrete the fusion protein, which improved the morphology of RGCs and decreased apoptotic signals. Specifically, IGF-1-tdTomato significantly increased the survival rate of RGCs, and enhanced both outgrowth and branching of axons. Our data indicate that IGF-1-tdTomato remarkably improves neuronal morphology, axonal growth and survival of RGCs compared to tdTomato (control) alone. Our findings primarily indicate that transfected hNPPFV cells can abundantly deliver IGF-1 and significantly invigorate neurons *in vitro*. This provides further evidence to support the application of hNPPFV cells as carriers of ectopic neurotophic factors for direct delivery to host RGCs under disease conditions.
* Corresponding author
1

T-3166

ENGRAFTED HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ANTERIOR SPECIFIED NEURAL PROGENITORS SUPPORT THE REPAIR OF THE RAT CRUSHED OPTIC NERVE

leila Satarian
Stem cell biology, Royan, Tehran, Iran, Islamic Republic of

Degeneration
of retinal ganglion cells (RGCs) is a common occurrence in several eye
diseases. This study examined the protection of RGCs in addition to the survival,
integration, differentiation, and functional repair capabilities of anterior

specified neural progenitors (NPs) which were produced under defined conditions from human induced pluripotent stem cells (hiPSCs) following intravitreal transplantation into rats whose optic nerves have been crushed (ONC). hiPSCs were induced to differentiate into anterior specified NPs expressing PAX6 and OTX2 by the use of Noggin and retinoic acid. The hiPSC-NPs were labeled by green fluorescent protein or DiI and injected into the vitreous cavity two days after induction of ONC in hooded rats. Functional analysis according to visual evoked potential recordings showed significant amplitude recovery in animals transplanted with hiPSC-NPs. Retrograde labeling by an intra-collicular DiI injection and counts of the labeled RGCs within the retina showed significantly higher numbers of RGCs and spared axons in ONC rats treated with hiPSC-NPs or their conditioned medium. hiPSC-NPs secreted neurotrophic factors that included ciliary neurotrophic factor, basic fibroblast growth factor, and insulin-like growth factor. Cell transplanted groups also had increased numbers of GAP43-positive axons and myelin staining by FluoroMyelin™ which imply for protection of
axons and myelin, and increased axonal regeneration. At 60 days post-transplantation hiPSC-NPs were integrated into the ganglion cell layer of the retina and expressed Brn3a, a RGCs marker. These results showed, the transplantion of anterior specified NPs in ONC rat model may improve optic nerve injury through neuroprotection, local RGC replacement, and optic nerve regeneration. These cells possibly provide a promising new therapeutic approach for traumatic optic nerve injuries and loss of RGCs caused by other diseases.

T-3167

THREE-DIMENSIONAL NEUROEPITHELIAL CULTURE FROM HUMAN EMBRYONIC STEM CELLS AND ITS USE FOR QUANTITATIVE CONVERSION TO RETINAL PIGMENT EPITHELIUM

Sven Schreiter[1], Yu Zhu[2], Madalena Carido[1], Andrea Meinhardt[1], Marius Ader[1], Elly Tanaka[1]
[1]Center for Regenerative Therapies Dresden, Technical University Dresden, Dresden, Germany, [2]Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Objective: We have developed a unique culture system to differentiate human embryonic stem cells (hESCs) quantitatively into retinal pigment epithelial (RPE) cells within 30 days (Zhu et al., PLoS ONE 2013). These cultures provide a good starting point to study important characteristics of RPE like phagocytosis ex vivo and represent a valuable tool that might be useful for medical and regenerative applications.
Methods and Results: The three dimensional (3D) culture conditions developed for MDCK epithelial cells were applied to hESCs in order to generate a polarized neuroepithelium. Within 24 hours after embedding hESC clumps in Matrigel the cells within the clumps reorganized into cyst-like structures containing a single lumen. After another five to six days of differentiation cysts uniformly express the transcription factors PAX6, RX and OTX2, suggesting eye field identity. Taking advantage of the speed and completeness of eye-field epithelia formation we showed that subsequent two-dimensional epithelial culture quantitatively differentiates into RPE within 30 days. We observed the appearance of pigmented RPE-like cells starting around day 18 with the characteristic polygonal shape emerging starting by day 25. The differentiated monolayer of RPE cells showed expression of typical RPE markers like RPE65, RX, MITF and Bestrophin. Ultrastructural analysis at day 50 confirmed the presence of tight junctions, desmosomes, microvilli and abundant melanin granules enriched toward the apical surface, which displayed the characteristic dome shape. To test the epithelial barrier function we measured the transepithelial resistance which gradually increased to 313 Ω cm-2. This correlates with the upper limit of human fetal RPE measured and suggests that the epithelial barrier function has been established. Finally we could show by negative stainings for 5-ethynyl-2’-deoxyuridine (EdU) that the RPE cells exited the cell cycle.
To demonstrate that our RPE cells were fully functional we investigated whether they support the uptake of shed outer segment disks of photoreceptors by phagocytosis - a key feature of RPE cells in vivo. Two approaches were

used: First, human ES cell-derived RPE-like cells were directly juxtaposed with transgenic mouse retina cells containing GFP outer segments structures. The detection of a GFP signal in the RPE cells confirmed that the RPE cells had phagocytosed shed outer segments. Second, using a rat model of retinal degeneration we could show that the RPE cells integrate after transplantation and form a donor-derived PRE monolayer in vivo that rescues photoreceptors.
Conclusions: This is the first time that a uniform RPE monolayer was created in vitro that closely resembles - both morphologically and functionally - RPE cells in vivo.

T-3168

TRANSIENT NEURONAL DIFFERENTIATION OF PUTATIVE PROGENITOR CELLS ISOLATED FROM THE ADULT HUMAN PERIPHERAL RETINA

Erik O. Johnsen, Agate Noer, Rebecca Frøen, Morten C. Moe
Oslo University Hospital, Oslo, Norway

Purpose: In adults, the retina is considered to have limited regenerative potential, and severe injuries lead to permanent damage. In cold-blooded vertebrates, however, retinal progenitor cells (RPCs) located in the ciliary marginal zone (CMZ) in the far peripheral retina can regenerate new retinal neurons throughout life. In addition, following damage, muller glia can re-enter the cell cycle and differentiate to replace lost neurons. Recently we reported cells positive for markers of RPCs around peripheral cysts of the adult human eye. These cells proliferated and migrated into the vitreous, generating nestin- and GFAP-positive sphere-like structures in patients with proliferative vitreoretinopathy (PVR). The objective of this study was to investigate the differentiation potential of cells isolated from the adult human peripheral retina.
Methods: Cells were isolated from the peripheral retina of patients undergoing surgery for PVR, as well as from post-mortem samples. These cells were cultured in neural stem cell medium for two passages before differentiation were induced on matrigel-covered flasks. Cells were analyzed using qPCR, ICC and Ca^{2+}-imaging.
Results and discussion: A significant upregulation of markers of differentiated photoreceptor cells, Crx, Rhodopsin and Recoverin, was detected both at the mRNA and protein level after 1 week of differentiation. However, the cells failed to maintain expression of these markers when cultured for a longer period of time (up to 5 weeks). These data indicate that putative retinal progenitor cells in the peripheral retina of the adult human eye have the ability to proliferate and express some RPC markers, but the differentiation potential towards neuronal cell types seem to be transient in vitro.

T-3171

PHOTORECEPTOR BEHAVIOUR DURING RETINAL DEGENERATION

Yvan Arsenijevic, Sarah Decembrini
Unit of Gene Therapy & Stem Cell Biology, Jules-Gonin Eye Hospital, Lausanne, Switzerland

Several
studies investigated whether Muller glial (MG) cells of the retina have a regenerative
potential to replace lost photoreceptors
during retinal degeneration. In *Zebrafish*, MG cells can replace
degenerating photoreceptors, whereas in mouse some MG cells start a
dedifferentiation program but do not complete the acquisition of a photoreceptor
fate. To date, few and unconfirmed studies addressed the photoreceptor behavior
during retinal degeneration. To that aim, we crossed the *Crx-GFP* with the *Glast-DsRed*
mouse lines expressing the green and red reporter genes in post-mitotic photoreceptors
and in adult MG cells respectively. We notably observed that during retinal
development the Glast transgene is expressed in retinal progenitors starting
from embryonic day (E) 14. Photoreceptor degeneration was induced by injecting the

neurotoxic MNU compound in 2 month old mice. Retinas of treated mice were collected after 1 day of treatment and embedded in culture to follow the expression of Crx and Glast transgenes by time lapse video. Using a fully automated cell tracking software, we traced migrating green and red fluorescent cells in time and space up to 2 days. Surprisingly, we observed that after treatment several displaced cells expressing GFP (post-mitotic photoreceptors) started translating the Glast-DsRed transgene.
So far, research focused prominently on the transition of glia towards a neuronal fate retinal degeneration and little attention was reported on photoreceptor behavior. The present data depict a new situation in which photoreceptors lose their specific identity and start expressing progenitor-like markers in the attempt to re-enter the cell cycle rendering the interpretation of regeneration, in certain situation, challenging.

T-3172

STROMAL STEM CELLS FROM LIMBAL BIOPSIES PROVIDE OPPORTUNITY FOR XENO-FREE AUTOLOGOUS THERAPY FOR CORNEAL BLINDNESS

Sayan Basu, Martha L. Funderburgh, James L. Funderburgh
Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA, United States

Purpose: Recent evidence suggests that multi-potent mesenchymal stem cells of neural crest origin are present in the anterior stromal layers of the limbus and peripheral cornea in humans. This study addresses the hypothesis that these superficially located stromal stem cells can be: a) isolated from clinically replicable limbal biopsies; b) expanded in xeno-free and potentially autologous culture conditions; and c) differentiated into corneal keratocytes.
Methods: One clock hour wide superficial stromal biopsies were obtained from the limbal region of human cadaveric corneo-scleral rims. Mesenchymal cells were isolated from the stroma by collagenase digestion of the limbal fragment, with or without epithelial removal by dispase, and cultured in media containing 2% bovine or human serum, with or without cholera toxin. At the third passage (P3) stromal cells were evaluated for: a) stem cell gene expression; b) clonal growth; c) sphere formation; d) expression of keratocytes marker genes in differentiation conditions; and e) organization of collagen lamellae on aligned nano-fiber substratum.
Results: Collagenase digestion without epithelial removal was the most efficient method of isolating stromal cells, which readily expanded in media containing human serum without cholera toxin. Epithelial cells were lost from the cultures by serial passage. P3 stromal cells had high clonogenecity compared to human fibroblasts, readily formed spheres and expressed stem cell gene markers (ABCG2, CXCR4, Nestin, NGFR, Oct4, PAX6 and SOX2). The stromal cells cultured using both bovine and human serum demonstrated similar ability to differentiate into functional keratocytes, characterized by up-regulation of keratocyte gene markers (ALDH3A1, AQPR1, CD34, Keratocan and PTGDS), secretion of keratan sulfate proteoglycans and generation of a multi-layered transparent matrix of aligned collagen fibers.
Conclusions: Ocular limbal biopsy is a potential source of autologous stromal stem cells. The ability of these stromal stem cells to differentiate into functional keratocytes opens the possibility of therapeutic strategies to cure blindness resulting from corneal stromal diseases with patient derived-cells isolated using autologous, xenobiotic-free conditions.

Neural Cells

T-3181

THREE-DIMENSIONAL NEURONAL TISSUE DEVELOPMENT FROM PLURIPOTENT STEM CELLS FOR DISEASE AND TOXICITY MODELS

Yannick Martinez[1], Sara Berzsenyi[1], Gabor Juhasz[2], Edina Varga[2], Viktor Szegedi[2], Karl-Heinz Krause[3], **Andras Dinnyes**[1]

[1]BioTalentum Ltd, Godollo, Hungary, [2]Institute of Biochemistry, Biological Research Center, Szeged, Hungary, [3]University of Geneva, Geneva, Switzerland

Modeling nervous system development, physiology and pathology in vitro is of paramount importance in the field of neuroscience. Traditional two-dimensional neuronal cultures offer relatively easy readouts for the level of differentiation, viability, toxicity and disease phenotypes; however, they do not provide enough complexity to mimic the in vivo structure and organization of the nervous system.
To overcome this barrier, three-dimensional neuronal models, such as engineered neuronal tissue (ENT), have been developed on air-liquid interface giving rise to a network of neurons and glial cells.
In our laboratory, we have adopted the technique of ENT differentiation from mouse embryonic stem cells. We cultured neuronal precursor cells on a hydrophilic membrane at air-liquid interface for one month and subjected the neuronal tissues to immunohistochemical, electronmicroscopic and electrophysiological analyses.
With immunolabeling of the cryosectioned 3D nervous tissue, we could detect Musashi1-, beta3tubulin-, MAP2-positive neuronal cells, among which some showed GAD67 or tyrosine-hydroxylase positivity, suggesting the presence of GABAergic and dopaminergic neurons. In addition, GFAP-reactive astrocytes emerged spontaneously in the 3D culture showing that the tissue is not composed of only neurons but other nervous system cell types are also present in the system.
Ultrastructural and immunohistochemical analyses revealed rosette-like organization of the neuronal precursor cells in the tissue and the differentiation of the cells in an inside-out manner, resembling the in vivo development of the neuronal progenitor cells.
Furthermore, we were able to detect biological electrical signals in the ENTs using multielectrode array (MEA). Pharmacological treatment of the tissue with action potential blocker (TTX) completely eliminated the signal, indicating that functional synapses are formed between the neurons.
After the complete standardization of the differentiation protocols, the engineered neuronal tissue model system might provide a useful tool for therapeutic target identification, low-throughput drug screening and hit validation.
This study was financed by EU FP7 (PartnErS, PIAP-GA-2008-218205; Resolve, FP7-Health-F4-2008-202047; STEMCAM; PIAP-GA-2009-251186).

T-3182

GLOBAL HYPO-METHYLATION ALTERS THE CELLULAR COMMITMENT OF CELLS DERIVED FROM PRIMARY CULTURES OF HUMAN FETAL NEURAL STEM CELLS.

Jose L. Roig-Lopez[1], Derry Alvarez[1], Karla Casillas-Pagan[1], Jose Monterrubio[1], Joshua Rosenthal[2], Jennifer E. Barreto[3], Sherly Pardo[4]

[1]School of Science and Technology, Universidad Del Este, AGMUS, Carolina, Puerto Rico, [2]Institute of Neurobiology, MSC, UPR, San Juan, Puerto Rico, [A]Anatomy and Neurobiology, [B]Biochemistry and Pediatrics, [3]Medical Sciences Campus, UPR, Rio Piedras, Puerto Rico

Changes in the epigenetic status of a stem cell modify the gene and protein expression profile that can result in an alteration in cell fate and determination. Primary cultures of human Neural Stem Cells (hNSC) from fetal tissue were used to study how *in vitro* demethylation influences the cellular commitment and differentiation towards neural or glial lineages. hNSC were characterized at the precursor stage and after differentiation. The derived cells were labelled with glial (GFAP) and neuronal markers (Beta tubulin III) to score the lineage commitment. Other markers such as O2 and Glutamine synthetase were used to verify lineage. Treatment with the hypomethylating agent 5-Aza-Deoxycytidine significantly decreased the percentage of neurons

derived from hNSCs. Neurons derived from both control and experimental hNSC groups had ionic currents characteristic of physiological bona fide neurons. No statistical difference was found for GFAP, however we observed a tendency towards a higher number of GFAP positive cells when treated with the hypomethylation agent. The results from these experiments will help predict changes in the commitment and differentiation of human neural precursor cells and could be applied to the development of cell replacement therapies.

T-3183

MODELING PATHOLOGICAL NEURO-GLIAL INTERACTIONS IN ADRENOLEUKODYSTROPHY.

Julien Muffat[1], Yun Li[1], Frank Soldner[1], Helen Bateup[2], Weifeng Xu[3], Bernardo Sabatini[2], Patrick Aubourg[4], Rudolf Jaenisch[1]

[1]Whitehead Institute for Biomedical Research, cambridge, MA, United States, [2]Harvard Medical School, Boston, MA, United States, [3]Picower Institute, cambridge, MA, United States, [4]Universite de Paris, PARIS, France

We created a human in vitro model of adrenoleukodystrophy (ALD), using iPS derivation as the basis. ALD is the prevalent monogenic demyelinating disorder, resulting from mutation of the peroxisomal fatty acid transporter ABCD1. Oligodendrocyte dysfunction triggers axonal degeneration, accompanied by astrocytic and microglial reactivity. ALD lacks a good animal model. It is an incurable disease, culminating in neuroinflammation and death in 60% of the patients. Death, but not handicap, can be prevented by bone marrow allograft and more recently by autologous transplant combined with gene therapy.
We generated iPS cells (hIPSCs) from 3 patients with known neuroinflammation, 3 patients with a non-inflammatory form, and 3 male controls. These lines were reverted to factor-free status and characterized for pluripotency. We optimized and developed differentiation and maintenance protocols for highly purified populations of the key cellular players, namely neurons, oligodendrocytes, astrocytes and microglia. Using combinations of cytokine-driven fate alteration, along with magnetic cell surface marker sorting to purify the cells of interest, we have identified culture conditions allowing long-term culture of these cell types. We have achieved significant myelination from oligodendrocyte precursors onto axonal segments, as monitored in live cultures using fluorescent dyes, as well as antibody staining followed by electron microscopy confirmation. These cultures have been analyzed for their canonical accumulation of very long chain fatty acids (VLCFA). Neuronal cultures have shown electrical activity on multi-electrode arrays, and by patch clamping. Mutant neuro-glial cultures are more sensitive than controls to stressors such as hydrogen peroxide and exogenous VLCFA. Also, we have identified defects in mitochondrial metabolism using plate-based respirometry. Given the peroxisomal nature of the disorder, and the mitochondrial involvement, we have generated mutant and wild-type cell lines carrying fluorescent organelle markers (including redox sensors). These will allow live monitoring of organelle biogenesis, fragmentation and transport, during long-term culture.
Mutations in the coding region of ABCD1 are fully penetrant, but the clinical presentation can vary widely, even for a given mutation. Owing to the complex effects of background on this monogenic disorder, we applied nuclease-enhanced gene targeting to generate isogenic pairs of wild type and mutant cells. Nucleases were generated to cut around the translation start site. Insertional null alleles have been identified in a wild-type male ES line. Conversely, a transgene overexpressing ABCD1 cDNA was transduced in an ABCD1 null iPS line. We are currently testing strategies to replace exon 1, which carries 30% of the mutations causing the disease.

T-3184

MYELIN INHIBITS THE FORMATION OF GFAP EXPRESSING AND LIF RESPONSIVE PRIMITIVE NEURAL STEM CELL COLONIES FROM THE ADULT SPINAL CORD

Wenjun Xu, Nadia Sachewsky, Cindi Morshead
Surgery, University of Toronto, Toronto, ON, Canada

Definitive neural stem cells (NSCs) in the adult forebrain comprise a subpopulation of GFAP expressing (GFAP+) subependymal (SE) cells. More recently we have demonstrated that the adult brain contains a population of LIF receptor positive (LIFR+)/ Oct4 positive (Oct4+) primitive NSCs (pNSCs) that are lineally related to the definitive GFAP+ NSCs. The adult derived pNSCs give rise to definitive NSCs that proliferate and generate progeny that migrate and differentiate into interneurons in the olfactory bulb. In vitro, adult derived LIFR+/ Oct4+ pNSCs and GFAP+ NSCs give rise to clonally derived, multipotent colonies of stem and progenitor cells. We asked whether the spinal cord also contains the definitive and primitive NSC populations and sought to characterize these populations in control and injury conditions. In a first set of experiments, we used GFAPtk transgenic mice that permit the selective ablation of proliferating GFAP+ cells in the presence of ganciclovir (GCV) to determine whether spinal cord NSCs express GFAP similar to their brain counterparts. Briefly, upon addition of GCV to primary or passaged spinal cord cultures we observed a complete loss of NSC colony formation from GFAPtk mice and no effect on the number of colonies from littermate controls. A similar result was observed following in vivo intraventricular administration of GCV for 3 or 5 days whereby no NSC colony formation was seen in vitro. Hence, definitive NSCs in the adult spinal cord express GFAP. In regard to the adult spinal cord derived pNSCs, we were unable to isolate this population from the spinal cord of wild-type mice in our initial assays. We hypothesized that (1) this was due to the rare nature of the pNSCs (~10 LIF colonies per brain from adult mice) and further speculated, based on initial observations, that (2) the presence of myelin in adult spinal cord cultures inhibited LIF colony formation. To address these possibilities we first looked for pNSCs from early postnatal spinal cords (postnatal day 1-7), a time when there is a 20X increase in the numbers of pNSCs in the brain and when there is significantly less myelin in the spinal cord. Indeed, we were able to isolate spinal cord pNSCs from these early postnatal pups. Based on these findings we attempted to isolate spinal cord pNSCs from adult shiverer -/- mice which are devoid of mature myelin basic protein and found that pNSCs colonies in LIF alone. We next performed co-culture experiments using wildtype YFP+ cells (from YFP expressing transgenic mice) with adult shiverer-/- cells and observed a significant reduction in the number of shiverer-/- derived definitive NSC colonies in the presence of myelin from YFP+ mice indicating that myelin has an inhibitory effect on colony formation. Indeed, the conditioned media derived from myelin containing primary cultures is sufficient to inhibit colony formation. Hence, the adult spinal cord contains a population of GFAP+ definitive NSCs and LIF responsive pNSCs, similar to what is observed in the adult brain. Finally, we have demonstrated that both the definitive and primitive stem cell populations are activated in response to minimal injury of the spinal cord.

T-3185

DIRECTED DIFFERENTIATION OF ADULT NEURAL STEM/PROGENITOR CELLS IN VIVO TO RESTORE HIPPOCAMPAL MYELINATION

Simon Braun[1], Raquel Machado[1], Nicolas Toni[3], Thorsten Buch[4], Burkhard Becher[2], Sebastian Jessberger[1]

[A]Brain Research Institute, [B]Institute of Experimental Immunology, [1]University of Zurich, Zurich, Switzerland, [2]Fundamental Neurosciences, University of Lausanne, Lausanne, Switzerland, [3]Institute for Medical Microbiology, Immunology and Hygiene, Technische Universität München, Munich, Germany

Neural stem/progenitor cells (NSPCs) generate new neurons throughout life in the mammalian brain. In the adult hippocampus, NSPCs give rise to granule cell neurons, however their fate is not restricted to the neuronal lineage. Retrovirus mediated overexpression of Ascl1 can direct the differentiation of NSPCs towards to oligodendrocyte lineage. In an inducible mouse model for demyelinating disease we evaluated the remyelination potential of NSPC-derived oligodendrocytes. To induce demyelination, we ablated oligodendrocytes in the adult hippocampus, using focal diphtheria toxin (DT) injections in transgenic mice that express DT receptors specifically in oligodendrocytes. We show that NSPC-derived oligodendrocytes mature in a demyelinated hippocampus and can contribute to remyelination. Furthermore, we show that other transcription factors besides Ascl1 are able to direct the fate of NSPCs towards the oligodendrocyte lineage in vivo. This approach highlights the potential of targeting endogenous NSPCs for the treatment of demyelinating diseases.

T-3186

TARGETING ADULT NEUROGENESIS TO PREVENT EPILEPSY

Jenny Hsieh
UT Southwestern Medical Ctr, Dallas, TX, United States

The capacity to produce new neurons throughout life with the demand of stem-cell maintenance is central to maintaining tissue homeostasis and preserving normal function during aging. However, under various pathological insults, resident neural stem cells can be hijacked to form abnormal circuits, which may cause, not prevent, epilepsy development. Clinical evidence showing hippocampal pathology and cognitive deficits in patients with temporal lobe epilepsy (TLE) suggests that increased hippocampal neurogenesis may be associated with epileptogenesis. Moreover, experimental epilepsy models demonstrate a dramatic increase of hippocampal neural stem cell proliferation and ectopic migration of newborn neurons with hilar basal dendrites and mossy fiber sprouting, which may contribute to spontaneous recurrent seizures (SRS). While previous attempts have been made to examine the role of seizure-generated granule neurons, it is not known if there is a strict cause-and-effect relationship due to the possibility of non-specific side effects. Therefore, to investigate the specific role of seizure-induced new neurons in epileptogenesis, we took advantage of a genetic approach and used a conditional transgenic mouse (Nestin-TK) to selectively delete dividing neural stem/progenitors and neuroblasts by ganciclovir (GCV) treatment. Four weeks of GCV administration led to >90% of doublecortin-positive adult-born neurons ablated in the dentate gyrus. Ten-week-old mice were administered pilocarpine to induce status epilepticus (SE). Only mice showing SE were selected for further evaluation of SRS. For a subset of animals, hippocampal cell death was confirmed at 7 days post-SE, showing pyknotic cells in the hilus, CA1 and CA3 subfield of the hippocampus. Seizure-induced animals showed SRS at 2 weeks post-SE by video and electrographic (video-EEG) monitoring. Comparison of EEG SRS between vehicle- and GCV-treated mice showed a 50% reduction of seizure frequency, but no change in the duration of seizures. These results support the finding that aberrant neurogenesis contributes to epileptogenesis, which may potentially open up new avenues for treating epilepsy.
NIH grants: R01NS076775, R01NS038572, and K02AG041815 to J.H. American Heart Association training grant 5T32HL007360-34 to Z.L.

T-3187

IMPAIRED NEUROGENESIS IN THE AGING FOREBRAIN: DEFINING THE KINETICS AND UNDERLYING MECHANISMS

Loïc M. Cochard, Laura K. Hamilton, Sandra E. Joppé, Anne Aumont, Karl J L Fernandes
Department of Pathology and Cell Biology, University of Montréal, Montréal, QC, Canada

In the adult brain, the subventricular zone (SVZ) surrounding the lateral ventricles is the principle site of neural precursor proliferation and adult neurogenesis. SVZ neurogenesis plays an important homeostatic role for forebrain neuronal and glial cell populations. However, for reasons that remain poorly understood, forebrain neurogenesis is highly diminished in the aged brain, likely contributing to reduced cognitive plasticity and regenerative potential. In this work, we aim to better understand the mechanisms involved in aging-associated reductions in adult neurogenesis. We began by performing a meta-analysis of the literature to determine whether consistent patterns of aging-associated changes could be identified. While this analysis revealed some common patterns of observations, it also identified major gaps in our knowledge. To begin filling these gaps, we undertook several lines of investigation. First, to more clearly establish the kinetics of aging-associated changes in SVZ neurogenesis, we performed a detailed time-course experiment spanning across key adult ages. Second, we used a variety of markers to assess the possible involvement of senescence-associated processes. Third, using biochemical and immunohistochemical approaches, we investigated changes in intracellular signaling pathways associated with neurogenesis reductions. We ultimately aim to test whether modulation of these signaling pathways can inhibit or prevent aging-associated neurogenesis impairments.

T-3188

NOREPINEPHRINE IS A NEGATIVE MODULATOR OF ADULT NEUROGENESIS

Grit Weselek[1,3], Andreas Herrmann[1,3,4], Silke Keiner[5], Torsten Christ[6], Barbara Kaltschmidt[7], Manfred Gerlach[8], Ursula Ravens[2], Christoph Redecker[5], Alexander Storch[1,3,4]

[A]Department of Neurology, Division of Neurodegenerative Diseases, [B]Department of Pharmacology and Toxicology, [1]Dresden University of Technology, Dresden, Germany, [2]Center For Regenerative Therapies, Dresden, Germany, [3]German Center for Neurodegenerative Diseases, Dresden, Germany, [4]Hans Berger Department of Neurology, Jena University Hospital, Jena, Germany, [5]Department of Experimental and Clinical Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [6]Department of Cell Biology and Molecular Neurobiology, University of Bielefeld, Bielefeld, Germany, [7]Department of Child and Youth Psychiatry and Psychotherapy, University of Würzburg, Würzburg, Germany

Adult neurogenesis takes place in two sites of the adult mammalian brain, the subventricular zone (SVZ) of the lateral ventricles and the hippocampal subgranular zone (SGZ). Even in the adult brain periventricular regions (PVRs) of the whole ventricular system harbour quiescent non-proliferative neuroblast-like neural progenitor cells (NPCs). Those cells can be isolated, propagated and differentiated into all major CNS cell types. These finding led to the hypothesis that local restrictive factors inhibit cell proliferation within the midbrain/hindbrain PVRs. Here, we identify the neurotransmitter norepinephrine (NE) as an endogenous negative modulator of adult neurogenesis. We show that in the adult brain in vivo NE levels are inversely correlated with cell proliferation in non-neurogenic PVRs and neurogenic SVZ. Furthermore, we performed pharmacological experiments in vitro as well as in vivo and demonstrate that NE inhibits NPC proliferation which in turns decreases SVZ-derived neurogenesis. Inhibitory effects of exogenous NE on neural stem and transient-amplifying cells in the SVZ are rescued by adrenoceptor antagonists. Moreover, application of different antagonistic mechanisms, blocking NE-mediated signalling and depletion of endogenous NE by selective degeneration of NE cells, results in an enhanced cell proliferation within non-neurogenic PVRs. In conclusion, our data provide novel insights in the mechanisms regulating NPC proliferation as initial step of neurogenesis in the adult mammalian brain and reveal NE as a negative regulator of adult ventricular-derived neurogenesis. Our results are of significance to establish novel therapeutic concepts for endogenous regeneration of neurodegenerative diseases located in regions nearby non-neurogenic PVRs such as the midbrain region in Parkinson's Disease.
Keywords: adult neurogenesis, norepinephrine, periventricular region

T-3191

THE ROLE OF NOTCH SIGNALING IN THE REGULATION OF NEURAL PROGENITOR CELLS IN THE ADULT HIPPOCAMPUS.

Fatih Semerci[1], William Tin-Shing Choi[1], Mirjana Maletic-Savatic[2]

[A]Developmental Biology, [B]Pediatrics, [1]Baylor College of Medicine, Houston, TX, United States

The unique capacity of adult hippocampus to generate new neurons throughout life is of great scientific and medical interest, as adult neurogenesis has been linked to learning and memory and a variety of neuropsychiatric disorders, including depression, epilepsy, and schizophrenia. However, the population of quiescent neural progenitors (QNPs), the main source of the new neurons, is restricted, which ultimately limits the total number of new neurons produced. Thus, investigations of the mechanisms that determine the proliferation capacity of QNPs and differentiation of secondary progenitors, amplifying neuroprogenitors (ANPs) are significant, as increasing their ability to produce newborn cells may have considerable implications for our ability to treat age-related disorders. Interestingly, both proliferation and asymmetric division of QNPs have been independently shown to be regulated by Notch signaling, but the role of Notch in controlling the ultimate fate of QNPs is unknown. We hypothesized that Notch signaling is necessary for the interaction of QNPs and ANPs, ultimately influencing the net contribution of QNPs to neurogenesis. To test this hypothesis, we first developed a reporter mouse line lunatic fringe (Lfng)-eGFP, which showed that Lfng is specifically expressed in QNPs. Lfng is one of the fringe modifiers of Notch and shifts Notch preference from Jag ligand to Dll ligand. Both Jag and Dll ligands are expressed in the dentate gyrus in the neuro-

genic niche. We further characterized the Lfng-eGFP mouse and determined that Lfng-eGFP positive cells gave progeny that followed the established neurogenic cascade, formed expected neural lineages in vitro, and responded to the electrical stimuli known to increase proliferation. In Lfng knockout mice, the number of QNPs and neuroblasts decreased significantly, strongly suggesting the functional role of Lfng in control of QNP behavior. These data solidified the Lfng-eGFP mouse as a new reporter mouse model for studies of primary adult stem cells in the hippocampus, QNPs. Further investigations of the Lfng role in adult neurogenesis will lead to better understanding of the regulation of stem cell proliferation by its progeny both in health and disease.

T-3192

AUTOCRINE REGULATION OF DENDRITE DEVELOPMENT IN ADULT NEWBORN NEURONS BY BDNF

Liang Wang, Liang She, Wei Huang, Mu-ming Poo
Institute of Neuroscience, Shanghai institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

In the mammalian brain, newly born neurons in the adult dentate gyrus are continuously being incorporated into the existing neural circuits. Newborn granule cells undergo several distinct developmental steps before becoming indistinguishable from mature granule cells, most of which were born during embryonic or early postnatal development. However, the molecular mechanisms regulating over this maturation process during adult neurogenesis remains largely unknown. In this study, we examined the potential autocrine function of brain-derived neurotrophic factor (BDNF) in the morphological maturation of newborn granule cells in the adult mouse hippocampus. By using retrovirus-mediated gene transduction, we found that deletion and overexpression of BDNF in single adult newborn neurons resulted in a reduction and elevation of dendrite growth of these neurons, respectively, as measured by total dendritic length and branch points. These results suggest that BDNF exerts autocrine action in the morphological maturation of these neurons. In BDNF conditional KO mice, where BDNF was deleted in all adult hippocampus, we found dendritic defects of adult newborn granule cells of similar extent as that found for BDNF deletion only in single newborn neurons, suggesting that paracrine contribution of BDNF may not be significant. Taken together, these results showed that BDNF autocrine action play an important role in regulating dendrite development in adult newborn neurons in the hippocampus.

T-3193

OCT4-EXPRESSING, LIF-RESPONSIVE, PRIMITIVE NEURAL STEM CELLS IN THE ADULT BRAIN ARE UPSTREAM OF THE GFAP POSITIVE DEFINITIVE NEURAL STEM CELL

Nadia Sachewsky[1], Wenjun Xu[1], Derek van der Kooy[2], Cindi M. Morshead[1]
[A]Surgery, [B]Molecular Genetics, [1]University of Toronto, Toronto, ON, Canada

Definitive neural stem cells (NSCs) in the adult forebrain comprise a subpopulation of GFAP-expressing subependymal (SE) cells. *In vivo*, GFAP+ adult NSCs proliferate and generate progeny that proliferate and migrate along the rostral migratory stream to the olfactory bulb where they differentiate into interneurons. *In vitro,* GFAP+ adult NSCs give rise to clonally derived colonies of stem and progenitor cells termed 'neurospheres' in the presence of EGF, FGF, and heparin (EFH). We have recently identified a novel LIF-responsive cell in the periventricular region of the adult forebrain that can generate clonal self-renewing, multipotent free-floating colonies *in vitro* in the presence of LIF alone, reminiscent of the LIF-responsive primitive NSC observed in the early embryonic brain. The adult derived LIF-responsive cell (AdpNSC) expresses the pluripotency marker Oct4 both *in vitro* and *in vivo*. We hypothesize that these AdpNSCs are lineally related to the GFAP+ adult NSCs. To definitively address the lineal relationship, we have utilized an ablation paradigm in GFAPtk/ROSAyfp mice that kills rapidly dividing progenitor cells (using the anti-mitotic agent AraC for 7 days) followed by infusion of ganciclovir (GCV) for 3 days to kill dividing GFAP+ cells. Exposure to Cre-recombinase retrovirus during the time of GCV infusion permits the labeling of non-GFAP expressing, LIF-responsive NSCs and an examination of their progeny over time. We observe YFP+ cells migrating towards the olfactory bulb *in vivo* 7 days post ablation, indicating that non-GFAP+ AdpNSCs proliferate and generate progeny to repopulate the adult germinal zone. Further, YFP+ LIF colonies and YFP+ EFH neurospheres

are observed *in vitro* at longer survival times but only YFP+ LIF colonies are observed at early times post-ablation. Similarly, when YFP/GFAPtk derived LIF colonies are grown *in vitro* in the presence of GCV (thereby eliminating GFAP+ adult NSCs within the colonies), collected and transplanted on the rostral SE, YFP+ cells are observed along the rostral migratory stream and in the olfactory bulb at 7 days post-transplantation. These findings reveal that AdpNSCs generate progeny that are capable of contributing to neurogenesis *in vivo*. Finally, Oct4-CreERT/ROSAyfp/GFAPtk mice reveal that AdpNSCs are Oct4 expressing and directly give rise to GFAP+ adult NSCs following tamoxifen recombination under baseline conditions. Together, these findings confirm the lineage relationship between the LIF-responsive AdpNSCs and the well characterized adult definitive NSCs and support the hypothesis that a LIF-responsive AdpNSC is upstream of the neurosphere-forming, GFAP+ adult NSC in the adult brain.

T-3194

PPM1D REGULATES WNT SIGNALLING AND ADULT NEUROGENESIS DURING AGEING

Yunhua Zhu
Institute of Molecular and Cell Biology, Singapore, Singapore

The number of new neurons generated from neural stem cells (NSCs) declines during ageing, which contributes to the functional deterioration of an aged brain. In turn, reduction of neurogenic capacity of NSCs could be due to certain intrinsic molecular change of NSCs that leads to inefficient commitment to neuronal lineage. Our data showed that the expression of Ppm1d in the mouse sub-ventricular zone decreased during ageing, and transgenic expression of Ppm1d rescued the age-related decline in neurogenic potential of NSCs in vitro. More importantly, new neuron formation in vivo and the deficit in olfactory function were both substantially rescued by transgenic Ppm1d expression in old mice. To find out the downstream molecular target of Ppm1d, we performed microarray screening and identified Dickkopf-3(Dkk3) as a novel target of Ppm1d. Interestingly, Dkk3 expression in neurogenic niche increased during ageing and was suppressed by Ppm1d expression. Deletion of Ppm1d in young NSCs lead to up-regulation of Dkk3 in a p53-dependent manner. Using Axin2-β-gal assay, we found that Dkk3 inhibited canonical Wnt signalling in NSCs, and specifically inhibit Wnt-induced neurogenesis through suppression of neuronal lineage commitment. Consistently, injection of a GSK3 inhibitor, which promotes canonical Wnt signalling, significantly rescued neurogenesis in aged mice. We propose that during ageing of NSCs, down-regulation of Ppm1d releases Dkk3-dependent suppression of Wnt signalling, and causes inefficient neuronal commitment.

T-3195

ALLOGENEIC TRANSPLANTATION OF NEURAL STEM OR PROGENITOR CELLS PROMOTED FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY IN NON-HUMAN PRIMATES

Hiroki Iwai[1], Hiroko Shimada[1], Soraya Nishimura[1], Yoshiomi Kobayashi[1], Tsunehiko Konomi[1], Osahiko Tsuji[3], Kanehiro Fujiyoshi[4], Keigo Hikishima[5], Yoshiaki Toyama[1], Masaya Nakamura[1], Hideyuki Okano[2]
[A]Orthopaedic Surgery, [B]Physiology, [1]Keio Univ, Tokyo, Japan, [2]Orthopaedic Surgery, Saitama Social Insurance Hospital, Urawa, Japan, [3]Orthopaedic Surgery, Murayama Medical Center, National Hospital Organization, Tokyo, Japan, [4]Central Institute for Experimental Animals, Kawasaki, Japan

Introduction: Several studies demonstrated that neural stem/progenitor cells (NS/PCs) promoted functional recovery in rodent animal models of spinal cord injury (SCI). However, there are many neuroanatomical and immunological differences between rodents and primates and thus it is critical to inspect allogeneic transplantation of NS/PCs in a non-human primate SCI model before clinical trials. The objective of the present study is to investigate the effectiveness and the safeness of allografted NS/PCs into injured spinal cord of common marmosets (Callithrix jacchus).
Materials and Methods: Cervical contusive SCI was induced using a modified NYU impactor in adult female common marmosets as reported previously . NS/PCs (1 million cells) derived from ganglionic eminence of a fetal marmoset were transplanted into the lesion epicenter at 14 days after SCI and an equivalent amount of PBS (phosphate

buffered saline) was injected instead of NS/PCs in the vehicle control group. In order especially to avoid acute rejection, the animals were subcutaneously administered with an immunosuppressant, FK506, at the dose of 1.0 mg/kg until 4 weeks post-transplantation and 0.1 mg/kg thereafter, daily. Motor function was assessed by original open field scoring scale, bar grip test and cage climbing test. Diffusion tensor tractography (DTT) was performed 1 week post-injury and 10 weeks post-transplantation and the number of tract fibers was quantified as reported previously. The spinal cord tissues were extracted 12 weeks after transplantation and processed for histological analyses.
Results: The transplantation group showed a better motor performance in the original open field score, grip strength and cage-climbing test score, compared to the vehicle control group. Sequential DTT revealed that the numbers of the tract fibers at 12 weeks post-SCI was significantly higher in the transplanted group than the control group. The grafted cells were well survived and differentiated into neurons, astrocytes and oligodendrocytes. NF-H (neurofilament 200 kDa), CaMK 2α (calcium/calmodulin-dependent protein kinase 2 alpha), PECAM-1 (platelet endothelial cells adhesion molecules-1) and HE (hematoxylin-eosin) positive areas, and LFB (luxol fast blue) positive myelinated areas were significantly larger in the transplantation group than the vehicle control group. There was no tumor formation in all the animals for 12 weeks after transplantation.
Discussion: Allografted NS/PCs were well survived without tumorigenesis and promoted functional recovery after SCI in common marmosets. Conventional MRI image has not been a helpful assessment method to reflect neurological function after SCI; however, this study suggests that DTT would be a clinically useful tool for assessment of NS/PC transplantation after SCI. This is the first study to report the efficacy and the safeness of the allogeneic NS/PC-transplantation for SCI in non-human primates.

T-3197

AMYOTROPHIC LATERAL SCLEROSIS MODEL CELLS DERIVED FROM HUMAN EMBRYONIC STEM CELLS OVEREXPRESSING MUTANT SOD1 OR TDP-43

Takehisa Isobe, Norie Tooi, Kazuhiro Aiba, Norio Nakatsuji
Institute for Integrated Cell-Material Sciences, Kyoto Univ, Kyoto, Japan

We previously reported a cellular ALS model derived from mutant SOD1-overexpressing hESCs. Our ALS model showed cellular phenotypes equivalent to ALS symptoms (Wada T et al, 2012). However, the integration site of mutant SOD1 was not defined in this model, and only single mutation type of SOD1 (G93A-SOD1) was used as a mutant SOD1 though more than 100 mutations are identified in SOD1.
In this study, to define the gene integration site and to compare mutation types of ALS-related genes in isogenic hESCs, we established mutant SOD1- or TDP-43-overexpressing hESC (mutSOD1-hESC or mutTDP43-hESC) lines were established by using the site-specific gene integration system (Sakurai K et al, 2009). A4V-, G85R- and G93A-SOD1, or D169G-, A315T- and M337V-TDP-43 were used as SOD1 or TDP-43 mutant types, respectively. The expression of exogenous SOD1 was driven by CAG promoter, while the expression of exogenous TDP-43 was regulated by the Tet-On system.
The morphology of mutSOD1-hESCs and mutTDP43-hESCs was normal like that of the parental cell line. Karyotype analysis showed that there were no chromosomal abnormalities in all of mutSOD1-hESCs. RT-PCR analysis showed that the expression level of endogenous SOD1 and TDP-43 was not affected by overexpression of exogenous genes in all of mutSOD1-hESCs and mutTDP43-hESCs, and that the expression levels of exogenous genes were almost equal among cells with the same mutant types. Increase of SOD enzyme activity was dependent on SOD1 mutations but the activity level was the same among the same mutant types. These data demonstrated that there were no clonal variations among hESCs with identical mutant types of mutSOD1 or mutTDP43. At present, we are carrying out motoneuron differentiation from mut-SOD1-hESCs and analyzing cellular phenotypes of hESC-derived motoneurons.

T-3198

THERAPEUTIC POTENTIAL OF IGF-I-MODIFIED HUMAN SPINAL CORD STEM CELLS AS A COMBINED THERAPY FOR ALS

Simon Lunn[1], Erika Sims[1], Crystal Pacut[1], Karl Johe[2], Eva L. Feldman[1]
[1]Dept of Neurology, University of Michigan, Ann Arbor, MI, United States, [2]Neuralstem Inc., Rockville, MD, United States

ALS is a fatal disease characterized by the loss of motor neurons. Currently there are no effective treatment options; however, the use of stem cells offers a new approach to treating this devastating disease. Stem cells may facilitate ALS treatment by providing a source of new neurons to augment neuronal circuitry or by providing trophic support to the existing cell population. We are currently assessing the therapeutic potential of human spinal cord stem cells (HSSC) in a phase I human clinical trial. Furthermore, we hypothesize that increasing the trophic support delivered by HSSC will improve their overall therapeutic impact. IGF-I is a neuroprotective factor known to promote neurite outgrowth and protect motor neurons. Therefore, we have engineered HSSC to overexpress IGF-I and compared the neuroprotective properties and efficacy of HSSC:IGF-I to their parental HSSC counterparts. We demonstrate that HSSC:IGF-I have enhanced neural differentiation and migratory potential compared to HSSCs. We also see no effect on terminal differentiation or the proliferative profiles of HSSC:IGF-I compared to HSSC. Finally, we demonstrate in vitro that HSSC:IGF-I have an increased ability to prevent glutamate induced primary motor neuron cell death. Furthermore, HSCC:IGF-I are more effective in protecting motor neurons in spinal cord organotypic cultures exposed to glutamate toxicity compared to parental HSSC. Together, our findings indicate that the combination of IGF-I with HSSC result in a more effective therapeutic option for therapeutic development for ALS

T-3201

CHARACTERIZATION OF AN IN VITRO MODEL FOR ALZHEIMER'S DISEASE USING NEURAL PRECURSOR CELLS

Daisyléa de S. Paiva[1], Simone A. A. Romariz[1], Gabriela F. Barnabe[2], Telma T. Schwindt[3], Beatriz M. Longo[1]
[1]Laboratory of Neurophysiology, Department of Physiology, Federal University of São Paulo, São Paulo, Brazil, [2]Ludwig Institute for Cancer Research, São Paulo, Brazil, [3]Institute of Biomedical Sciences, Department of Developmental and Cell Biology, University of São Paulo, São Paulo, Brazil

In vitro studies of embryonic neural precursor cells (eNPC) obtained from fetal telencephalon have contributed to better understanding the basic mechanisms of neural diseases. Because of their ability to proliferate in culture, the eNPC can be used as strategy for studying proliferation, migration and neural differentiation. In order to reproduce the characteristics observed in patients with Alzheimer's disease(AD), various transgenic models expressing Amyloid Precursor Protein (APP) were developed. In the double transgenic APPswe/PS1dE9 mice, the clinical symptoms usually start to appear slowly, starting around 4 months of age. *In vitro* studies with eNPC may contribute to investigate the cellular aspects of the disease, helping to clarify some important features difficult to explore in *in vivo* models. Precursor cells harvested from the telencephalic vesicles of APPswe/PS1dE9 (AD) and wild-type (WT) mice embryos were cultured as floating neurospheres. Culture media was composed by DMEM/F-12, 2 mM L-glutamine, 20 ng/ml of FGF-2, 20 ng/ml of EGF, and 2% B-27 at 37 °C in 95% humidity and 5% CO_2. In order to evaluate the effect of AD in the growth rates, the area of individual neurospheres were measured each 3 days for a period of 15 days. We found that neurospheres from AD animals were larger than WT ones only at day 6 ($p<0.05$), but there was no difference among the groups at any other evaluated time points (3, 9, 12 and 15 days)(Unpaired t test with Welch's correction). For studies on migration and cell fate determination, neurospheres were allowed to attach to poly-L-lysine and laminin-coated coverslips and the growth factors were removed from media composition to trigger the differentiation of neuropheres for 10 days. The migration assay was evaluated as the distance of the foremost cells to the neurosphere boundary. No difference in migration was found among the groups (Unpaired t test, $p>0.1$). Imunofluorescence was performed in plated neurospheres with primary antibodies against β-tubulinIII, Nestin, GFAP and Amyloid-β 42 (6E10), markers of neurons, neural precursors, astrocytes and amyloid plaques, respectively. In a qualitative analysis, despite both groups expressed neural markers (Nestin, GFAP, β-tubulinIII) after differentiation, only the AD group stained for 6E10, indicative of amyloid-β 42 presence, when compared to WT. Within the neurospheres, transgenic cells differentiated preferably in astrocytes, while the majority of WT cells was positive for Nestin. It is noteworthy that the morphology of both Nestin-positive and GFAP-positive

cells that carrying alterations is different due to cytoplasm elongation and large nuclei. The WT group presented radial glia-like phenotype, with large processes and thinner cells than AD ones. eNPC of both groups were able to differentiate into neurons. The effect of early amyloid-β 42 expression in the functionality and plasticity of these neurons will be discussed in future experiments. Although in animal models of AD the amyloid plaques start to be frequent in the brain at 4 months of age, in AD neurospheres amyloid plaques appear in the neurogenic phase, indicating that the development of the pathology occurs early in the embryo and can be validated as a platform for studying the cellular and molecular mechanisms present in AD.

T-3202

CELLULAR ALZHEIMER'S DISEASE MODELS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS OVEREXPRESSING MUTANT PRESENILIN 1

Kazuhiro Aiba[1], Itsunari Minami[1], Norie Tooi[1], Makoto Honda[2], Norio Nakatsuji[1]
[1]Inst for Integrated Cell-Material Sciences, Kyoto Univ, Kyoto, Japan, [2]Stem Cell and Drug Discovery Institute, Kyoto, Japan

Recently we have developed an efficient system to integrate a gene of interest into a defined locus in the genome of hPSC. Using this site-specific gene integration system, we established hESC and hiPSC lines carrying mutant presenilin 1 (PS1) responsible for familial Alzheimer's disease (AD) to investigate molecular mechanisms of AD progression.
Overexpression of mutant PS1 did not affect undifferentiated state, chromosome stability and neural differentiation of hESCs. In addition, there were no differences in action potential amplitude and frequency of neurons derived from parent hESCs and hESCs overexpressing wild-type or mutant PS1. However, mutant PS1 overexpression resulted in decrease of spontaneous excitatory post-synaptic current as well as increase of Aβ42 and Aβ43 production. These results might be equivalent to cellular phenotypes of AD symptoms, suggesting that genetically manipulated hPSC-derived disease model cells would be useful tools for AD research.

T-3203

DIFFERENTIATION OF HUMAN IPSCS TO ROSTRAL VERSUS CAUDAL NEURONAL FATES REVEALS FATE-SPECIFIC ALZHEIMER'S DISEASE RELEVANT PHENOTYPES

Christina Muratore, Heather C. Rice, Priya Srikanth, Meichen Liao, **Tracy L. Young-Pearse**
Neurology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, United States

Alzheimer's disease (AD) is characterized by extracellular plaques containing amyloid β-protein (Aβ) and intracellular tangles containing hyperphosphorylated Tau protein. Distinct brain regions are differentially susceptible to neurodegeneration in AD, with the cortex and hippocampus being primarily affected and the cerebellum and spinal cord being relatively spared. We describe the generation of inducible pluripotent stem cells (iPSCs) from humans harboring the London fAD APP mutation (V717I), and examine AD-relevant phenotypes following directed differentiation of these cells to rostral forebrain neuronal fates vulnerable in AD, as well as more caudal fates, which are relatively spared in AD. Immunostaining, qPCR, Nanostring analyses and Western blotting were used to examine expression of factors central to APP cleavage. ELISAs and Western blotting were utilized to measure the generation and secretion of APP cleavage products, as well as expression and phosphorylation of Tau. In both rostral and caudally directed neuronal cultures, the APP V717I mutation led to elevated production of Aβ42 and Aβ38. Unexpectedly, this mutation, which lies near the γ-secretase cleavage site in the transmembrane domain of APP, also led to increased β-secretase cleavage of APP. This increase was abolished by inhibiting γ-secretase, suggesting that the two protease activities are functionally linked. Directing cells to different neuronal fates resulted in altered cleavage patterns of APP. From both control and fAD lines, Aβ generated from neurons directed to forebrain cortical fates showed a higher Aβ42 to Aβ40 ratio relative to neurons directed to more caudal fates of the hindbrain and spinal cord. Moreover, consistent with fate-specific biochemical effects on Aβ, the APP V717I mutation led to increased Tau expression in forebrain cultures but not in more caudally directed neurons. These studies identify previously

unappreciated effects of an fAD APP mutation in human neurons and demonstrate that human iPSC technology provides a powerful system for analyzing the effects of both genetic alterations and cell types on processes directly relevant to human brain diseases.

T-3204

ADULT HIPPOCAMPAL NEUROGENESIS IN THE VENTRAL HIPPOCAMPUS IMPROVES BEHAVIORAL SYMPTOMS IN AN ALZHEIMER'S DISEASE MODEL

Daniel Offen, Adi Shruster
Tel Aviv Univ, Peta-Tikva, Israel

Alzheimer's disease (AD), the most common form of dementia and cognitive decline, is a devastating illness, with one in eight people over the age of 65 having the disease. Non-cognitive symptoms, termed the Behavioral and psychological symptoms of dementia (BPSD), are frequently observed and affect 80% of patients. These include depression, disinhibition, delusions, hallucinations, agitation, anxiety and aggression. AD has been associated with impaired neurogenesis in the hippocampal dentate gyrus, which is linked to cognitive impairment along with several behavioral and psychiatric disorders.
Previous studies have shown that Wnt/beta-Catenin Signaling pathway promotes baseline neurogenesis in the adult hippocampus. To investigate the effect of neurogenesis on behavioral function in a relevant disease model, Wnt3a gene is delivered bilaterally to the dentate gyrus in the ventral hippocampus of 3xtg-AD mouse model via lentivirus (LV-Wnt3a). Animals injected with LV-Wnt3a at a presymptomatic stage show significantly improved neurogenesis and behavioral symptoms. Wnt3a treatment increased behavioral inhibition and risk assessment in the 3xtgAD model, as measured by the emergence test, elevated plus maze, open field and rat exposure test. This effect was blocked by ablation of neurogenesis with X-irradiation.
Thus, our data indicate that targeting adult neurogenesis in the ventral hippocampus has potential as an alternative therapy of the behavioral changes in AD and possibly other neurodegenerative disorders.

T-3205

TRAUMATIC BRAIN INJURY REVEALS NOVEL CELL LINEAGE RELATIONSHIPS WITHIN THE SUBVENTRICULAR ZONE.

Janel Le Belle[1], Gretchen Miller-Thomsen[2], Neil Harris[2], Harley Kornblum[3]
[A]Psychiatry, [B]Neurosurgery, [C]Psychiatry and Biobehavioral Sciences, Pharmacology and Pediatrics, [1]UCLA, Los Angeles, CA, United States

The acute response of the rodent subventricular zone (SVZ) to brain injury involves a physical expansion through increased cell proliferation but the cellular underpinnings of these changes are not well understood. Our analyses have revealed that there are two distinct transit-amplifying cell populations in the SVZ that respond in opposite ways to injury. Mash1+ transit-amplifying cells are the primary SVZ cell type that is stimulated to divide following brain injury and these cells account for the majority of the proliferative response. In contrast, the EGFR+ population which has been considered to be a functionally equivalent transit-amplifying progenitor population to Mash1+ cells in the uninjured brain becomes significantly less proliferative after injury. While transit amplifying cells are thought to derive from a population of slowly dividing GFAP+ stem cells, the GFAP+ stem cells themselves are not stimulated to divide more after injury. We found, instead, that brain injury results in increased numbers of GFAP+/EGFR+ stem cells via non-proliferative means_potentially through the dedifferentiation of progenitor cells. In vitro EGFR+ progenitors from injured brain, but not control brains were competent to revert to a stem cell state following brief exposure to growth factors. Thus, our results demonstrate previously unknown changes in lineage relationships that differ from conventional models of lineage progression based on cell ablation experiments and likely reflect an adaptive response of the SVZ to maintain the endogenous brain repair processes after traumatic brain injury.

T-3206

ROLE OF C-FOS DURING NEUROGENESIS

Fabiola Noelia Velazquez[1], Diego Sergio D'Astolfo[2], David Silvestre[3], Olivier Etienne[4], François Boussin[4], Beatriz Leonor Caputto[1]

[1]Departamento de Química Biológica, CIQUIBIC-CONICET, Córdoba, Argentina, [2]Utecht University, Utrecht, Netherlands, [3]Institut Curie, Paris, France, [4]Laboratoire de RadioPathologie-IRCM, CEA, Fontenay-aux-Roses, France

The *c-fos* proto-oncogen is an immediate early gene, with a characteristic rapid transcriptional activation in quiescent cells following mitogenic stimulation. Constitutive *c-fos* expression is seen in a limited number of tissues, however, including amniotic and placental tissue, fetal liver, adult bone marrow and growing bone, and in the developing central nervous system. *c-fos -/-* mice showed a significant decrease in viability and those who survive birth growth at a normal rate until ten days of age, subsequently they develop a decrease in the growth rate, osteopetrosis, absence of gametogenesis, lymphopenia, among other disorders. They have also altered behavior including hyperactivity and a decreased response to external stimuli suggesting some alteration in neural function. The *c-fos* proto-oncogene is expressed in many situations involving neuronal differentiation and stimulation, which suggests that the c-Fos protein plays a role in regulation of the genes involved in neuronal organization and function, such as those encoding cytoskeletal proteins, enzymes and neurotransmitters.
Neurons of the mammalian central nervous system originate from progenitors dividing at the apical surface of the neuroepithelium. These cells show a high capacity of proliferation, and an adequate control of their growth and differentiation capacity is of key importance. We proposed to determine if the absence of c-Fos has an effect during neurogenesis. First we evaluated the comportment of neural stem progenitor/cells using *in vitro* assays. Neuronal Colony Forming Cell (NCFC) assays showed that *c-fos-/-* embryos (at 14.5 days (E14.5) have three times less neural stem cells in the telencephalon than *c-fos+/+* embryos (% of neural stem cells/total cells: *c-fos-/-* 1,02%; *c-fos+/+* 2,53%). Measure of proliferation of neurospheres cultures, through H_3-Tymidine incorporation at different time points, showed differences in the kinetic proliferation between neural stem/progenitor *c-fos -/-* and *c-fos +/+* cells, without significant differences in apoptosis. Then, through *in vivo* assays we compared the development cerebral cortex on day 14.5 of *c-fos+/+* and *c-fos-/-* mice. Assays of dual injection of EdU and BrdU in E14.5 *c-fos-/-* and *c-fos+/+* showed an increased number of cells at the S-phase in the embryonic cortical telencephalon of *c-fos-/-* mice. Immunofluorescence assay showed c-Fos expression at the cortical level while different markers of terminally differentiated neurons (BIII-Tubulin, NeuN and Tbr1) indicated less number of differentiated cells in the *c-fos-/-* mice. Primary cortical culture in presence of NGF shows a lower percentage of neuronal differentiation in the *c-fos-/-* mice than in the *wt* condition. Determination of the mitosis angle in apical progenitors reflected a predominant symmetric division in the knock out condition. These results indicate that the absence of *c-fos* affects: the average cell-cycle length of progenitor cells (causing an exhausting of these cells) and/or the differentiation capacity of them (avoiding retire from the undifferentiated steady).

T-3207

CHEMOTATIC EFFECT OF CXCL12 AND PK2 ON ADULT NEURAL STEM CELL-DERIVED NEUROBLASTS

Mayara T V Vieira-Mundim, Layla T. Galindo, Marimélia Porcionatto
Biochemistry, UNIFESP, São Paulo, Brazil

There are two well-defined neurogenic areas in the adult mammal brain: the subgranular zone of the hippocampus, which cells integrate the granular zone of the dentate gyrus, and the subventricular zone, an area that produces neuroblasts that migrate through the rostral migratory stream to the olfactory bulb, where they differentiate into interneurons. However, injuries to the central nervous system stimulate neuroblasts to migrate from the neurogenic niche in the subventricular zone, guided by chemoattractive molecules produced at the injured site. Additionally, injury leads to the production of axonal growth inhibitory molecules, such as chondroitin sulfate proteoglycans and formation of the glial scar. Our aim was to investigate the migratory behavior of neuroblasts under stimuli, prokineticin 2 (PK2) that mimics the olfactory bulb environment, and CXCL12, that mimics an injury site environment. Using neural stem cells (NSC) cultured as neurospheres submitted to concentration gradients of both chemotactic factors,

we observed increased distance of migration of NSC towards PK2 and CXCL12 comparing to the migration of cells away from the source of each chemoattractant. When neurospheres were submitted to both stimuli, the neuroblasts migrated longer distances towards CXCL12 when compared to the distances migrated towards the source of PK2. However, culturing neurospheres in the presence of chondroitin sulfate led to the inhibition of neuroblast migration, even in the presence of CXCL12. Our data suggest that CXCL12 stimulus is more attractive to neuroblasts than PK2, although its chemoattractive effect is not sufficient to revert the inhibitory effect of chondroitin sulfate.

T-3208

EPIGENETIC ACTIVATION OF NEURONAL GENE EXPRESSION BY JMJD3 IS REQUIRED FOR POSTNATAL BRAIN NEUROGENESIS

Dae Hwi Park, Gabriel Gonzales-Roybal, Sung J. Hong, Shawn Sun, Jacopo Sgualdino, Giuseppe Testa, Martin M. Matzuk, Naoki Iwamori, **Daniel Lim**
UCSF, San Francisco, CA, United States

While it is now well known that new neurons are born in the subventricular zone (SVZ) of the adult mammalian brain, the epigenetic mechanisms that enable lifelong neurogenesis in SVZ neural stem cells are poorly understood. Here we show that JMJD3, a histone H3-lysine 27 demethylase, is required for SVZ neurogenesis in young adult mice. JMJD3 is expressed in adult SVZ neural stem cells and is upregulated during neuronal differentiation. Conditional knockout of Jmjd3 in SVZ neural precursors inhibits the postnatal production of neurons for the olfactory bulb, and immunohistochemical analysis of the SVZ germinal region suggests that Jmjd3-null neural stem cells fail to efficiently generate young migratory neuroblasts. In vitro, Jmjd3-deficient neural precursor cells survive and proliferate, but neuronal differentiation is greatly impaired. During neurogenesis, JMJD3 becomes enriched at the intergenic enhancer of Dlx2, a critical neurogenic transcription factor, and our chromatin immunoprecipitation data indicate that Jmjd3 is required to activate this enhancer for proper Dlx2 expression. Without Jmjd3, this enhancer remains ‘poised’ with high levels of H3K27me3, and Dlx2-dependent neurogenesis fails. Our data thus reveal a crucial role for JMJD3 in the genesis of neurons in the postnatal brain and furthermore provide novel mechanistic insight into how key transcriptional enhancers are activated during lineage specification.

T-3211

C-MYC AND ADULT NEUROGENESIS

Ahmet M. Denli, Chunmei Zhao, Mohamedi Kagalwala, Iryna Gallina, Tithi Baul, Fred H. Gage
Salk Institute, La Jolla, CA, United States

Stem cells play an important role not only during development but also in maintenance of the adult organisms. Adult stem cells are found in most, if not all organs including the brain, which was once thought to be devoid of neurogenic activity.
Adult neurogenesis is a process through which new neurons are born in the adult brain with potential links to learning and memory as well as neurological disorders. Neurogenesis takes place in the subgranular zone of the dentate gyrus (DG) and the subventricular zone of the lateral ventricles. In the DG, newborn neurons mature to become excitatory granule cells that integrate into the existing circuitry. Adult neurogenesis is fueled by relatively quiescent radial glial cells, which give rise to a proliferative cell population, most of which adopt a neuronal cell fate. While the cell types involved in adult neurogenesis have been characterized, we are far from a full understanding of molecular mechanisms involved.
The highly conserved transcriptional regulator c-myc is an integral part of development and also acts as a proto-oncogene. While a large number of studies have focused on c-myc, only a minority has been carried out in normal adult stem cells. A clear picture of c-myc function remains to emerge, due to context-dependence, overlapping expression patterns with other myc proteins, weak transcriptional activity and low expression levels in adult cells. c-myc is involved in diverse processes such as cell growth, proliferation, apoptosis and differentiation: phenomena intimately linked to neural stem cell biology.

We have hypothesized that c-myc may have a function in early steps of adult neurogenesis. We first confirmed expression of c-myc in the adult DG as well as cultured adult hippocampal progenitor cells (AHPs). c-myc bound to its known target loci and regulated expression in AHPs, suggesting that c-myc was functional in this cellular context. Expression of c-myc was downregulated upon differentiation both in vivo and in vitro. Knockdown of c-myc led to enhanced differentiation, whereas overexpression of c-myc inhibited differentiation of AHPs. Retroviral expression of c-myc in the DG led to an early increase in proliferation followed by an inhibition of subsequent differentiation. Interestingly, further analysis suggested that c-myc function was dose-dependent and that c-myc might have a direct role in early differentiation.
Overall, our data point to a novel role for c-myc in regulation of not only cell proliferation but also cell fate in adult neurogenesis.

T-3212

DIVERSE REGULATION OF CORTICAL LAMINAR IDENTITY OG HESC-DERIVED NEURONS BY SOX5

Radmila Filipovic, Saranya Santosh Kumar, Joseph Loturco
PNB, University of Connecticut, Storrs, CT, United States

Previous studies demonstrated that Sox5 controls postmitotically
the timing of layer 5 neurons by suppressing Ctip2 expression in deep layer neurons
in rodents. Depending of reports, misexpression of Sox5 either converted upper
layer neurons to deep layer neurons, or was not sufficient to induce aberrant
projections of these neurons. However, it is not known how transgene expression
of Sox5 affects distinctive stages of neuronal development in humans. In present studies we introduced Sox5 transgene
in either early, late human neuronal progenitor cells (hNPs), or in induced
neurons, using stable integration of transgenes by *PiggyBac* (PB) transposon system and studied the generation of deep
layer specific neurons. hNPs were derived from H9 hESC (Stem Cell core, UCHC,
Farmington, CT) and isolated from the center of rosettes at two stages of
development, at 15 days in vitro (div, early hNP) or 21 div (late hNP) and
transfected with either pPBCAG-Sox5, or pPB-CAG plasmids along with pCAG-PBase.
Transfected cells were fixed after one week and stained with markers of layer
VI neurons (Tbr1) or layer V neurons (Ctip2). Our results show that in hNPs
forced expression of Sox5 has a diverse effect on two stages of cells: in early
hNPs Sox5 transgenesis decreased two times the percentage of corticothalamic
neurons (Tbr1+) and increased the percentage of Ctip2+ subcerebral neurons
three times. However, when Sox5 expression was increased by transgenesis in
late progenitors, it did not significantly change the percentages of Tbr1 and
Ctip2 neurons, consistent with temporal specificity of Sox5 effects. Finally,
when hESCs were transfected with Sox5 in combination of neuronal- inducing
transcription factors (Brn2, Ascl1 and Myt1l; BAM pool), Sox5 transgenesis increased
the percentage of Tbr1 (2.5 times), while reduced Ctip2 (3 times) percentage in
MAP2+ induced neurons three weeks after induction, as compared to control (BAM).
In conclusion, Sox5 induces progression of early, but not late hNPs to deep
layer neurons.

T-3213

SPECIFIC GENERATION OF ENRICHED PV VERSUS SST EXPRESSING INTERNEURONS FROM A DUAL-REPORTER MESC LINE

Jennifer A. Tyson[1], David Tischfield[1], Asif M. Maroof[2], Stewart A. Anderson[1]

[1]Psychiatry, Children's Hospital of Philadelphia, Philadelphia, PA, United States, [2]HSCI, Harvard, Boston, MA, United States

Abstract: GABAergic cortical interneurons originating in the medial ganglionic eminence (MGE) express either the Ca2+-binding protein parvalbumin (PV) or the neuropeptide somatostatin (SST) upon maturation. It is the morphology, connective profiles and individual firing patterns unique to each subgroup that provides the crucial inhibitory input for normal cortical function. Deficits in both PV and SST fate specification have been differentially implicated in the etiology of a variety of neurological diseases such as epilepsy and schizophrenia. Furthermore, these cells have the remarkable ability to extensively migrate and survive after transplantation into postnatal cortex, making them attractive candidates for use in cell-based therapeutics. Towards this end, generating a limitless population of distinct interneuronal subgroups is of great translational value. Two lineage-defining transcription factors crucial to PV and SST interneuron development are; Nkx2.1, which is expressed by progenitors then immediately down regulated and Lhx6, which is expressed as these cells become post mitotic and is maintained through adulthood. SST interneurons preferentially originate early in the dorsal MGE (dMGE), whereas PV interneuron generation peaks in the ventral MGE (vMGE) later in embryonic neurogenesis. This study describes an efficient protocol to generate interneurons of a ventral telencephalic lineage as evidenced by the robust induction of Foxg1, Nkx2.1 and Lhx6. We have built an Nkx2.1::mCherry-Lhx6::GFP dual reporter mESC line to isolate cortical interneuron progenitors versus postmitotic cells of the same lineage. Additionally, for the first time we demonstrate the ability to differentially purify enriched populations of PV versus SST expressing cells through recapitulating developmental programs in vitro. This is accomplished through FACS isolating dMGE-like early born cells differentiated with high levels of sonic hedgehog (SHH) to enrich for SST, or alternatively by FACS isolating vMGE-like later born cells differentiated without exogenous SHH to enrich for PV+ mESC derived cortical interneurons. Finally, we are also in the early stages of exploring the utility of each enriched population as a potential cell based therapy for seizure disorders through collaborations with expert physician scientists at CHOP. In addition, the identification of early genetic markers of PV-fated cells will be a tremendous resource for future fate determination and function studies of this essential subgroup of interneurons. This work is funded by R01 MH066912-01 and T32 1T32HD060600.

T-3214

PROTEOLYTIC PROCESSING OF CXCL12 DISRUPTS MIGRATION AND VIABILITY OF ADULT MICE NEURAL STEM CELLS IN VITRO

Taís Adelita de A Barros[1], Roberta S. Stilhano[2], Sang W. Han[2], Giovani B. Peres[1], Giselle Z. Justo[3], Marimélia Porcionatto[1]

[A]Biochemistry, [B]CTCMol, [C]Biological Science, [1]UNIFESP, São Paulo, Brazil

The chemotactic factor stromal cell-derived factor 1 (SDF1/CXCL12) and its receptor CXCR4 are expressed in several tissues including the CNS. During development, homeostasis, injury and neurodegenerative diseases the CXCL12-CXCR4 axis is responsible for stimulating migration, proliferation and survival of several cell types including endogenous neural stem cells (eNSC) from the subventricular zone. CXCL12 N-terminal can be cleaved by matrix metalloproteinases 2 and 9 (MMP2/9) generating a new molecule, CXCL12(5-67), with poorly understood effects on eNSC. MMP2/9 are found in pro-death microenvironments present in neurodegeneration and brain injury. This work focuses on investigating if MMP2/9-cleaved form of CXCL12 is involved in eNPC survival. In order to do that, we produced recombinant CXCL12(5-67), and evaluated its effect on mice NSC chemotaxis and viability *in vitro*. CXCL12 and CXCL12(5-67) were cloned into uP vector that was previously constructed by inserting a sequence of DNA containing cytomegalovirus (CMV) intron 1 with splicing signals between the CMV promoter and polycloning site of pVAX. Hek293T cells were transiently transfected and secreted CXCL12 and CXCL12(5-67) were quantified by ELISA. CXCL12 and CXCL12(5-67) chemotactic activity was assessed by transwell chemotaxis assay using $CXCR4^{+}$ Jurkat cells. The native form of CXCL12 was able to stimulate Jurkat cells chemotaxis, whereas CXCL12(5-67) did not promote chemotaxis. Likewise, only CXCL12 was able to improve NSC migration out of neurospheres in an adherent cell migration assay. Viability of NSC, assessed by Alamar Blue assay, was reduced by treatment with CXCL12(5-67)

when compared to cells treated with CXCL12. In conclusion, we observed that the partially cleaved form of CXCL12, CXCL12(5-67), does not show chemotactic activity and decreases viability of NSC *in vitro*.

T-3215

MACHINE LEARNING BASED IMAGE ANALYSIS FOR THE AUTOMATED COUNTING OF MOTOR NEURONS IN A LIVE CELL SCREENING SYSTEM WITHOUT THE USE OF SPECIALIZED FLUORESCENT PROBES

Samuel V. Alworth[1], Andre' Blumenstein[2], Anthony C. Arvanites[2], Justin Ichida[2], Chieko Nakada[3], Yasujiro Kiyota[3], Kevin Eggan[2], Lee L. Rubin[2], James S.J. Lee[1]

[1]DRVision Technologies LLC, Bellevue, WA, United States, [2]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States, [3]Nikon Corporation, Tokyo, Japan

There has been a great deal of recent interest in using transcription factor mediated direct conversion of fibroblasts into differentiated cells. Using this method for certain applications, such as image-based screening, is dependent on the ability to readily recognize the differentiated cells. This is often achieved using a lentiviral vector to deliver a differentiation-activated fluorescent reporter. For example, we have produced multiple lines of spinal cord motor neurons using a set of 6 transcription factors (Son et al., Cell Stem Cell, 2011, 9:205) and a lentiviral Hb9::RFP reporter. However, we have found that there can be background expression of RFP in cells other than motor neurons, and it is useful to define motor neurons on the basis of other structural features, such as the presence of neurites. Here we report our validation of an innovative image analysis recipe (processing routine) using CL-Quant image recognition software (Nikon Corporation). The recipe incorporates two levels of machine learning. The first is done at the image level to enhance the fine neuronal processes, making use of a teachable machine learning method called soft matching. And the second is done at the feature level, creating motor neuron classifiers from the cell measurements and uses a teachable machine learning method called regulated decision trees. The method achieves a high accuracy and good correlation with biological controls. Going forward, we will create a live cell screening assay based on the machine learning methods that can be used for large-scale neuronal survival assays.
Supported in part by grant number 4R44HL106863 from the NHLBI, K award number K99NS077435-01A1 from the NIH to JI, and the Harvard Stem Cell Institute.

T-3216

UNIQUE ROLE OF DOPAMINE RECEPTORS IN DIFFERENTIATION FROM PLURIPOTENT STEM CELLS

Yusuke Hamada[1], Naoko Kuzumaki[2], Yukari Suda[1], Michiko Narita[1], Moe Watanabe[1], Eri Shimura[1], Chizuru Iwasawa[1], Yohei Okada[2], Wado Akamatsu[2], Hideyuki Okano[2], Minoru Narita[1]

[1]Pharmacology, Hoshi University, Tokyo, Japan, [2]Physiology, Keio University, Tokyo, Japan

It has been reported that monoamines and neuropeptides appear in the embryo before cell differentiation, and have functions during embryogenesis such as differentiation and growth. In the present study, we investigated the role of dopamine in ES and iPS cell differentiation. At first, we found the progressive expression of many dopamine-related genes, such as tyrosine hydroxylase, dopamine receptors and dopamine transporters, from undifferentiated ES and iPS cells to differentiated neural stem cells (NSC), while the mRNA of monoamine metabolic enzymes was high dense even in ES and iPS cells. Interestingly, the temporal profiles of D2 and D3 receptor mRNAs were opposite to each other. The D2 receptor expression was progressively increased, whereas D3 receptor expression appeared to decline with the course of differentiation from ES and iPS cells. Next, we profiled the influence of dopamine receptor stimulation located on ES and iPS cells on expressing the GPCR in NSCs. To profile the expression of mRNAs encoding 384 GPCRs inNSCs, a TaqMan GPCR-specific microarray analysis was performed. A heat-map of the microarray revealed considerable differences in the expression of GPCRs between dopamine receptor-stimulated NSCs by dopamine and non-stimulated cells. These findings support the possibility that dopamine may play a role in regulating ES/iPS cell differentiation and transforming the differentiated NSCs with changing the expression of GPCRs.

T-3217

THE ROLE OF CASPASE 9 INHIBITOR ON DOPAMINERGIC NEURONAL DIFFERENTIATION FROM ORGANOTYPIC CULTURED MEDIUM PRETREATED HUMAN NEURAL STEM CELLS

Hsiao-Nan Hao[1], Jean Peduzzi[2], Peter Hao[1], BeiZhong Xie[3], Yunlin Tang[4], Huizi Yang[5]
[A]Orthopedic surg, [B]Anatomy and cell biology, [1]Wayne State University, Detroit, MI, United States, [2]Xiamen Neocell Biotech, Xieman, China, [A]Neurosurg, [B]OB/GYN, [3]PLA 458 Hospital, Guangzhou, China

Introduction: Commitment of human neural stem cell to differentiate into dopaminergic neuronal cells is an essential step to pursue cell therapy for the Parkinson's disorders. The dopaminergic neuronal differentiation (DND) requests many endogens and exogenous steps such as intracellular signals. The inhibition of apoptosis has shown the regulatory function to anticipate the DND. Inhibition of caspase 9 signals may have similar function of BcL2 to enhance DND. Using human neural stem cell (hNSC) cultures, we are able to observe the DND differentiation after hNSC treated with pGB caspase 9 siRNA vector (BioVision). The DND can be directly induced from hNSC by transfection of pGB caspase 9 SiRNA vector. The similar results were found after the hNSC treated with caspase 9 inhibitor peptides VX-765. However, the rate of DND is around 4-6% with caspase 9 inhibition. To increase the DND rate, the hNSCs were cultured with condition medium which was generated from human fetal brain oganotypic culture. It is likely that the combination of VX-765 and the condition medium increases the DND rate significantly. Although the mechanism remains to be explored, the caspase 9 inhibition associates with RhoA/RAK activation which involves DND.
Experimental Results: The purpose of this study was to determine if inhibition of caspase 9 regulates DND from primary hNSC cultures. The gene expression profiles of DND generated from hNSC (nestin+/CD133+) were analyzed using cDNA microarray representing 11000 mRNAs. The significant alterations of RhoA/RAK expression were confirmed by RT-PCR, Northern blotting and protein assays. The levels of OCT4, SOX2 and Nonog were estimated after hNSC were treated with and without caspase inhibitor. Compared with non-treated cultures, RhoA/RAK genes were up-regulated respectively (4 to 7 times respectively) with the inhibitor. Both OCT4 and SOX2 expressions increase following elevated Rho/RAK expression. Dopaminergic neuronal markers (such as TH, Hu, Neu-N, NF-L, N-cadherin, β-III Tubulin and MAP-2) were positively detected in the condition medium and inhibitor treated cultures. TH positive cells occupied about 21% ± 5 of DCD cultures. Dopamine concentration was measurable. In contrast, non-treated cultures did not show neuronal marker expression. This data suggest that the inhibition of apoptotic caspase pathway may play a critical role on DND via RhoA/RAK signals.
Material Methods: Protocols for human fetal brain tissue culture, cell isolation, and primary cell culture have been proved by HIC of university and hospital authorities. The condition medium was prepared from human fetal brain oganotypic culture. The caspase 9 siRNA vector and peptides VX-765 were purchased from commercial available. miRNA expression profiling of both hNSC and dopaminergic differentiation analyzed use micro-array. Total RNA samples were extracted from untreated NSCs, cells cultured within serum-free condition medium for 7 days, then cells treated with caspase-9 inhibitor and vector for 3 days. The transcription levels of nestin and neuronal specific protein makers were analyzed by reverse transcriptase (RT)-PCR and immunocytochemistry stains. Dopamine concentration was estimated use HPLC.

T-3218

THE TRANSCRIPTION FACTOR PROTEIN ROADMAP OF HUMAN FETAL NEOCORTICAL AND STRIATAL DEVELOPMENT

Valentina Castiglioni[1], Marco Onorati[1], Romina Vuono[2], Rocio Laguna Goya[2], Roger Barker[2], Elena Cattaneo[1]
[1]Department of Biosciences and Centre for Stem Cells Research, University of MIlan, Milan, Italy, [2]Cambridge Centre for Brain Repair, University of Cambridge, Cambridge, United Kingdom

The proper functioning of the vertebrate central nervous system depends on the correct assembly of neural circuits containing the appropriate types of neurons produced in the right numbers, at the right time, and in the right place.

In the telencephalon, this assembly is achieved by coordinated control of patterning, proliferation and differentiation of neural progenitors. In the embryonic telencephalon, the cerebral neocortex and striatum develop largely from two adjacent neurogenic territories along the dorsal-ventral axis: the pallium dorsally and the lateral ganglionic eminence (LGE) ventrally, respectively.
However, very little is known about the specific transcription factors that participate in the definition of these domains in the developing human brain. Our aim here is to obtain a comprehensive and precise topographical benchmark of the developing neocortex and striatum at different stages of human embryonic and fetal life starting from 2.5 weeks until 11 gestational weeks.
Through immunodetection analyses we assessed the expression of 12 transcription factors, together with striatal and cortical specific antigens. We show that OTX2 and FOXG1 are espressed from 3 weeks of gestation in the closing anterior neural tube and later identify the telencephalic ventricular and subventricular zones (VZ/SVZ) both in the pallial and subpallial regions. In the developing neocortex PAX6 - one of the earliest neuroectodermal markers espressed at the neural fold stage - and TBR2- that identifies cortical neural progenitors- are expressed in the VZ and SVZ respectively, while TBR1, CTIP2 and V-Glut are expressed in the differentiated cortical plate starting from 7 gestational weeks. In the VZ of the LGE, GSX2 expression seems characterized by a dorsal (high) to medial (low) gradient, as observed in rodents. A convergence of antigenic features instead identifies the striatal progenitor transition through the SVZ, while discrete areas in the LGE mantle zone are characterized by the emergence of differentiating striatal projection neurons expressing post-mitotic markers such as FOXP1, FOXP2, CTIP2, DARPP-32 and GABA starting from 7 weeks of gestation.
Through this work we expect to identify the precise set of co-expressed transcription factors and markers that uniquely identify the neural progenitors and neural precursors as well as the maturing neurons of the developing human neocortex and striatum. We also expect that this work will facilitate studies aimed at the generation of authentic striatal and cortical neurons from human pluripotent stem cells.

T-3221

GENOME-WIDE TRANSCRIPTOMIC ANALYSIS OF HUMAN NEOCORTICAL AND STRIATAL DEVELOPMENT

Marco Onorati[1], Valentina Castiglioni[1], Romina Vuono[2], Ramesh Menon[4], Daniele Biasci[3], Cinthia Farina[4], Gianvito Martino[4], Rocio Laguna Goya[2], Roger A. Barker[2], Elena Cattaneo[1]
[1]University of Milan, Milan, Italy, [A]Cambridge Centre for Brain Repair, [B]Cambridge Institute for Medical Research, [2]University of Cambridge, Cambridge, United Kingdom, [3]San Raffaele Scientific Institute, Milan, Italy

Human neurodevelopment occurs through a complex and finely regulated program that depends on a precise regulation of gene expression. To gain a global molecular perspective of the transcriptome in the developing human telencephalon, we explored the spatio-temporal dynamics of gene expression in human neocortical and striatal areas from embryonic to early fetal periods. In particular, we used genome-wide gene expression analysis in order to define a precise signature, based on typical and novel classes of genes, that distinguishes the different populations of developing neocortical and striatal regions. An unsupervised systematic analysis was performed on neocortical and striatal samples and hierarchical clustering of differentially expressed genes segregated the two telencephalic transcriptional programs. As a parallel approach, we used Sparse Subspace Clustering to identify a minimal set of transcriptions factors and cell fate determinants that are able to classify stage-specific neurodevelopment-associated transcriptional neocortical and striatal programs. In a third approach, we investigated the telencephalic gene expression organization through systematic analysis of gene coexpression relationships, in order to identify modules of coexpressed genes that distinguish the transcriptional program of the developing neocortex and striatum. This study provides a comprehensive data set on the human telencephalic transcriptome architecture during a pivotal, but previously poorly recognized, neurodevelopmental period.
We also expect that this work will facilitate studies aimed at the generation of fully functional, authentic striatal and cortical neurons from human pluripotent stem cells.

T-3222

FUNCTIONAL BENCHMARKING OF HUMAN FETUS-DERIVED CORTICAL AND STRIATAL PRIMARY NEURONS

Gerardo R. Biella[1], Marco Onorati[2], Elisabetta Cesana[1], Valentina Castiglioni[2], Romina Vuono[3], Mauro Toselli[1], Roger A. Barker[3], Elena Cattaneo[2]

[1]Biology and Biotechnologies, University of Pavia, Pavia, Italy, [2]Biosciences and Center for Stem Cell Research, University of Milan, Milan, Italy, [3]Centre for Brain Repair, University of Cambridge, Cambridge, United Kingdom

Here we explored the in vitro neurogenic potential of neural stem/progenitor cells (NSCs/NPCs) isolated from the developing neocortex and striatum of human fetuses, aged 7-9 weeks post-conception and immediately subjected to neuronal differentiation. We demonstrated that, under optimized monolayer cell culture conditions, NSCs/NPCs maintain positional identities consistent with the region of origin. Furthermore, we show that human fetus-derived NSCs/NPCs promptly differentiate into mature cortical and striatal neurons expressing a set of region-specific cell determinants and a spectrum of neurochemical markers as well as previously uncharacterized functional properties. In particular, after a 32-day long differentiation protocol, neocortical NSCs/NPCs give rise to glutamatergic pyramidal neurons, while striatal NSCs/NPCs differentiate into both projection medium spiny neurons (MSNs) and local interneurons.

Electrophysiological characterization of young human neurons is currently very limited. We now report that resting membrane potentials are rather depolarized in all immature (-45±12 vs 26±3 mV; mean ±s.e; striatal vs cortical) and fully differentiated (39± 4 vs 49 ±5 mV) human neurons. Accordingly, no spontaneous action potentials have been recorded. Furthermore, as expected, the amplitude of the membrane capacitive current increased during differentiation whereas the values of the input resistance decreased.

A distinctive feature of fully differentiated neurons is their ability to fire action potentials. Whereas K+ channels (KDR) current was expressed in all cells and its amplitude did not change during differentiation, the percentage of neurons expressing a peak of INa greater than 500 pA increased significantly during differentiation (33% vs. 52% and 25% vs. 78%; immature vs. fully differentiated; striatal and cortical neurons, respectively). These results were consistent with the repetitive action potentials firing elicited in mature neurons following stimulation with supratreshold rectangular depolarizing pulses.

The differentiation towards a fully mature neuronal phenotype also implies that these neurons should be able to develop a functional synaptic network. Under voltage-clamp conditions we established that the totality of cortical and striatal fully differentiated neurons expressed functional NBQX-sensitive glutamatergic and bicuculline-sensitive GABAergic receptors.

Altogether these data demonstrate for the first time the suitability of human primary neuronal cultures to functional benchmarking. We also expect that this work will facilitate studies aimed at the generation of fully functional, authentic striatal and cortical neurons from human pluripotent stem cells.

T-3223

IDENTIFYING THE NEURAL STEM CELL HIERARCHY IN HUMAN FETAL BRAIN AND HIGH GRADE GLIOMA

Siddhartha S. Mitra[1], Sharareh Gholamin[2], Abdullah Feroze[1], Samuel H. Cheshier[2], Irving Weissman[1]

[A]Institute of Stem Cell Biology and Regenerative Medicine, [B]Neurosurgery, [1]Stanford University School of Medicine, Stanford, CA, United States

The understanding of human fetal brain development, has been severely restricted due to the lack of identifying markers which can distinguish between human central nervous system (CNS) neural stem cells (huNSC) and CNS multi-potent progenitor cells (huCNS-MPPs or NPCs). The specific identification of live huNSC has been compounded not only due to the lack of known surface markers but also selective reporters for these cells. Furthermore our understanding of developmental processes at various stages of gestation and their effect on intrinsic lineage commitment is at present incomplete hence the need to reproducible identify these cells becomes ever more important. Here we present our results on identifying and characterizing the surface markers to prospectively isolate and separate huNSCs and huCNS-MPPs from various gestational stages of human fetal brain using multi color flow-

cytometry. After screening a panel of 34 antibodies to prospectively isolate neurospheres forming cells from human fetal brain tissue we narrowed down to a permutation of four antibodies (i.e CD15, Notch1, EGFR, and CD90) which marked the NSC/NPCs cells and further four antigens which marks either non-CNS lineage cells (i.e CD45, CD31 and CD34) or differentiated CNS lineage cells (CD24) Subpopulations of cells derived after FACS were analyzed for single cell lineage potency, self renewal and clonogenic characteristics. Using an in-vitro limiting dilution assay, we show that double-positive (DP; CD15+Notch1+EGFR-CD90-) populations have the highest frequency of self-renewing cells, followed by quadruple-positive (QP) populations (CD15+Notch1+EGFR-CD90-). qPCR analysis and In-vitro differentiation assays reveal both DP and QP to be multipotent in nature however serial passaging showed that only the DP cells have a consistent self-renewing capacity, whereas QP derived cells were unable to self renew after 5-6 passages. Further more single cell differentiation assays from sorted fetal brain cells at various stages of gestation (9-22 weeks) shows a switch between neurogenic to astrogenic potential at 14 to 16 week of gestation with the first evidence of oligodendrocytes at approximately 18 weeks. Currently we are in the process of carrying out in-vivo lineage analysis of the sorted populations. In glioma samples however we observed by limiting dilution and serial passaging assays that the tumorsphere forming frequency was predominant in the QP population and not in the DP populations. Furthermore the GBM derived DP cells serially passaged if grown under normal neural stem cell growth condition suggesting that this population is still at a pre-malignant state. In-vivo tumorigenic assay via orthotopic implation of as few as a 1000 cells into mouse brain showed significantly higher tumor formation from the QP cells and not in the DP cells. Taken together our data suggests the presence of “normal” or pre-malignant stem cells in glioblastoma, which suggests a possible mechanism of relapse. These pre-malignant cells could comprise a cellular reservoir that may need to be targeted for future therapies.

T-3224

AMNIOTIC FLUID STEM CELLS WITH LOW γ-INTERFERON RESPONSE ALLEVIATED PARKINSONISM IN RAT MODEL

Ming-Song Tsai[1], Yu-Chen Chang[2], Tsung-Yen Ho[3], Mei-Ling Wu[2], Tzyy-Wen Chiou[3], **Shiaw-Min Hwang**[2]
[1]Prenatal Diagnosis center, Cathay General Hospital, Taipei, Taiwan, [2]Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan, [3]Department of Life Science, National Dong Hwa University, Hualien, Taiwan

Amniotic fluid stem cells (AFSCs) are multipotent stem cells that may be used in clinical cell therapy. In this study, AFSCs established from amniocentesis were further investigated for translational medicine in Parkinson’s disease. We examined the cell surface expression of human leukocyte antigens (HLA) in these cells and estimated the therapeutic effect of AFSCs in parkinsonian rats. The expression profiles of HLA-II and transcription factors were compared between AFSCs and bone marrow-derived mesenchymal stem cells (BMMSCs) with γ-IFN treatment. The results showed that CIITA and RFX5 were weakly induced by γ-IFN stimulation in AFSCs compared to that of BMMSCs. Consequently, stimulation of AFSCs with γ-IFN prompted only a slight increase in the expression of HLA-Ia and HLA-E, and the expression of HLA-II remained undetectable. In transplantation test, Sprague-Dawley rats with 6-hydroxydopamine lesioning in the substantia nigra were used as a parkinsonian-animal model. We found that the apomorphine-induced rotation rate was reduced by 75% in AFSCs engrafted parkinsonian rats but was increased by 53% in the control group by 12-weeks post-transplantation. The injected AFSCs were viable, migrated into the brain’s circuitry and expressed specific proteins of dopamine neurons, such as tyrosine hydroxylase and dopamine transporter. We concluded that AFSCs might have immune-tolerance in γ-IFN inflammatory condition, and the effective improvement of AFSCs transplantation paves the way for the clinical application in parkinsonian therapy.

T-3225

THE LAMININ ALPHA-1 CHAIN IS INVOLVED IN THE CONTROL OF NEURAL STEM CELLS PROLIFERATION AND DIFFERENTIATION

Dominique Bagnard[1], Lionel Meyer[1], Céline Heng[2], Olivier Lefebvre[1], Jürgen Bolz[2]

[1]MN3T Lab, INSERM U1109, Strasbourg, France, [2]Institute of General Zoology and Animal Physiology, University Friedrich-Schiller of Jena, Jena, Germany

The molecular mechanisms orchestrating the construction of the cerebral cortex are still poorly understood. Here, we focused on the potential involvement of extracellular matrix heterotrimeric glycoproteins of the Laminin family due to their well-established role in cell migration and differentiation in various organs. In particular, we developed a conditional knockout (cKO) mouse of the gene coding for the Laminin alpha 1 chain (Lama1). We discovered that Lama1 deficient mice exhibit severe cortical dysplasia associated with lateral ventricles dilatation. Histological and functional in vivo assays revealed that Lama1 absence leads to abnormal positioning of Cajal Retzius cells, defects in radial glia and disorganized migration of deep layer neurons. Strikingly, we showed increased proliferation of neural progenitors in Lama1 deficient E15 mice embryos exhibiting a 3-fold increase in BrdU uptake when compared to WT embryos from the same litter. The analysis of the proliferative capacity of Neural Stem Cells (NSC) grown in the form of neurospheres (NS) produced from WT of cKO mice also revealed a 1.6 fold increase of NSC proliferation in Lama1 deficient mice. Intriguingly, we also noticed overproduction of oligodendrocytes when differentiating NSC on a Poly-ornithine substrate. Hence, we conclude that Lama1 has a key role in cortical development inherent to effects on NSC proliferation, neuronal migration and differentiation. Future studies will investigate how these early developmental defects may be associated with cognitive impairments.

T-3226

EZH2 REGULATES NEURAL STEM CELL FATES IN THE DEVELOPING MOUSE MIDBRAIN

Martina Zemke[1], Kalina Draganova[1], Haruhiko Koseki[2], Lukas Sommer[1]
[1]Institute of Anatomy, University of Zürich, Zürich, Switzerland, [2]RIKEN Research Center for Allergy and Immunology, RIKEN Yokohama Institute, Yokohama, Japan

The size and growth of the developing brain is determined by the choice of multipotent neural stem cells (NSC) between self-renewal and differentiation. The polycomb group protein Ezh2, enhancer of Zeste homolog 2, is the catalytic subunit of polycomb repressive complex 2 (PCR2) and is primarily responsible for trimethylation of histone H3K27 (H3K27me3). This epigenetic mark contributes to repression of many genes, which are pivotal for neural development. Here we show that Ezh2 is essential for the regulation of neural progenitor (NP) fate decision in the developing mouse midbrain. Wnt1-Cre-mediated ablation of Ezh2 results in a shortened caudal midbrain caused by impaired NP proliferation from embryonic day (E) 12.5 onwards. In addition mutant NP exit the cell cycle precociously resulting in increased neural differentiation. Intriguingly mutant midbrains show a downregulation of pro-proliferative Wnt/β-catenin signaling. To identify direct Ezh2 repression targets we performed whole-genome transcriptome analysis of mutant and control midbrains as well as H3K27me3 ChIP. Together our data suggest a role of Ezh2 in regulating NSC fate decisions by suppression of Wnt/β-catenin signaling in the developing midbrain.

T-3227

CD200 AND HLA-A,B,C ENABLE THE ISOLATION OF NEURONS FROM NEURAL INDUCTION CULTURES OF HUMAN EMBRYONIC STEM CELLS.

Jason Vidal, Nil Emre, Jody Martin, Christian T. Carson
BD Biosciences, La Jolla, CA, United States

The differentiation of human pluripotent stem cells to neural ectoderm presents an opportunity to study human neurogenesis and neurodegenerative diseases. Previous work has demonstrated that it is possible to identify distinct cell populations that represent developmental transition points in neural induction cultures using cell surface markers and fluorescence activated cell sorting (FACS). To identify additional cell surface signatures of neural cell types we combined intracellular marker expression with cell surface immunophenotyping. We performed a large unbiased screen of 242 antibodies that recognize cell surface epitopes while analyzing for intracellular expression of neural stem cell markers Pax6, Sox1 and Sox2 and the neuronal marker doublecortin (DCX). A comparative analysis of cell populations defined by the expression of Pax6, Sox1 and DCX revealed that the surface markers HLA-A,B,C, CD340, CD49f, and CD151 were expressed on DCX- cells while CD200 differentially stained DCX+ cells from other cells in culture. FACS of CD200++/HLA-A,B,C- cells from neural induction cultures, containing 15% neurons resulted in 90% pure population of neurons. This methodology was compared with currently published methods that enable the sorting of neurons from cultures of neural stem cells that have been differentiated to neurons. Magnetically depleting the neuron induction cultures with antibodies against HLA-A,B,C, CD340 and CD49f enriched these cultures four to five fold for neurons. These methods enable enrichment of neuronal cells from heterogeneous cultures for subsequent downstream analysis.

T-3228

TARGETED QUANTITATIVE AMNIOTIC CELL PROFILING: A POTENTIAL DIAGNOSTIC TOOL IN THE PRENATAL MANAGEMENT OF NEURAL TUBE DEFECTS

Elliot C. Pennington, Fabienne L. Gray, Azra Ahmed, David Zurakowski, Dario O. Fauza
Surgery, Boston Children's Hospital, Boston, MA, United States

Purpose: Neural tube defects (NTDs) can pose significant diagnostic and ethical dilemmas during the prenatal period, particularly in their more severe forms, when termination of pregnancy may be justified. A more precise diagnosis can occasionally be challenging, typically very early in gestation. We hypothesized that targeted quantitative analyses of select cell populations within fresh, unprocessed samples of amniotic fluid may correlate with NTD type and/or size. If so, this could contribute to the differential diagnosis and management of certain cases of NTDs prenatally. In this study, we sought to test this hypothesis in a chemically-induced model of NTDs.
Methods: After IACUC approval, 10 time-dated pregnant Sprague-Dawley dams underwent prenatal exposure to 60mg/kg of all-trans retinoic acid on embryonic day 10 for the induction of fetal NTDs. Animals were killed before term for analyses, at which time amniotic fluid samples from all fetuses were procured prior to scrutiny for the presence, type, location, and size of NTDs. Samples from 61 fetuses could be analyzed. Each individual fresh amniotic sample (volume = 0.2-0.7mL) underwent quantitative multicolor flow cytometry for the detection of cells concomitantly expressing Nestin and Sox-2, primary markers of neural stem cells (aNSCs), as well as of cells concomitantly expressing CD29 and CD44, markers of mesenchymal stem cells (aMSCs). Data were expressed as the ratio of positive cells to the overall number of cells. Statistical analysis included ANOVA and post-hoc Bonferroni adjusted comparisons, with significance set at $p<0.05$.
Results: A NTD was present in 57% (35/61) of the fetuses, namely either an isolated spina bifida (n=19), an isolated exencephaly (n=6), or a combination of the two (n=10). There was a statistically significant increase in the proportion of aNSCs in fetuses with spina bifida (6.78±1.87%), when compared with fetuses with exencephaly (0.64±0.23%), and with fetuses with both spina bifida and exencephaly (0.22±0.09%). Conversely, there was a statistically significant decrease in the proportion of aMSCs in fetuses with exencephaly, either isolated (1.09±0.42%), or in combination defects (2.37±0.63%), when compared with normal fetuses (8.83±1.38%). No such differences were noted in fetuses with isolated spina bifida. In fetuses with isolated exencephaly, there was a statistically significant inverse correlation between the proportion of aNSCs and defect size. This did not apply to fetuses with spina bifida, or combination defects.
Conclusions: The proportion of neural and mesenchymal stem cells in the amniotic fluid correlate with the type and size of select neural tube defects. Quantitative amniotic cell profiling may become a useful diagnostic tool in the prenatal management of these anomalies. Further analyzes of this concept in neural tube defects, as well as possibly other congenital anomalies, is warranted.

T-3231

FGF1 DEPENDENT AURORA A KINASE ACTIVITY SUSTAINS THE SELF RENEWAL AND MULTIPOTENCY OF NEURAL STEM CELLS AND GLIOBLASTOMA STEM CELLS

Yi-Chao Hsu[1], CHIEN-YU KAO[2], Yu-Fen Chung[2], ING-MING CHIU[2]

[1]Institute of Biomedical Sciences, Mackay Medical College, New Taipei City, Taiwan, [2]Institue of Cellular and System Medicine, National Health Research Institutes, Jhunan, Taiwan

Glioblastoma (GBM) comprises a subpopulation of tumor-initiating cells that display characteristics of stem cells (GBM-SCs), which have been suggested as an important target of anti-GBM treatment. Recent evidence suggests that mitotic kinase Aurora-A (AurA) sustains the embryonic stem cell (ESC)-like characteristics. Activation of AurA is not only associated with genome instability in cancer, but also regulates critical processes in asymmetrical division of stem cells. In this study, we provide several lines of evidence to demonstrate that AurA activation (Thr288 phosphorylation) is crucial for the maintenance of neural stem/progenitor cells (NSPCs) and GBM-SCs: (i) Inhibition of AurA activation reduced the self-renewal of mouse ESCs, mouse ESC-derived NSPCs, primary mouse brain NSPCs and human GBM-SCs. (ii) FGF-1B promoter (-540 to +31)-driven green fluorescent protein (F1BGFP) facilitates the isolation of F1BGFP(+) cells with higher levels of AurA activation and pluripotency marker expression, such as NANOG, OCT4 and SOX2, from human glioblastoma cell line, U-1240 MG. (iii) F1BGFP reporter could identify GBM-SCs with higher AurA kinase activation, self-renewal capacity and multipotency from human glioblastoma tissues. (iv) Inhibition of AurA activity by two different AurA inhibitors not only reduced neurosphere formation but also promoted neuronal differentiation of F1BGFP(+) GBM-SCs. Taken together, F1BGFP facilitates the isolation of GBM-SCs with higher AurA activation, self-renewal and multipotency. FGF1-dependent AurA kinase activity is crucial for the maintenance of human GBM-SCs and thus a potential target for cancer stem cell-directed therapy of GBM.

T-3232

EFFECTS OF BDNF ON THE DIFFERENTIATION OF MOUSE NEURAL PROGENITORS LACKING FRAGILE X MENTAL RETARDATION PROTEIN

Maija Liisa Castren, Venkat Swaroop Achuta, Veronika Rezov, Lauri Louhivuori, Karl Åkerman
Institute of Biomedicine, Univ of Helsinki, Helsinki, Finland

Fragile X syndrome (FXS) is a common cause of inherited intellectual disability and the best characterized form of autistic spectrum disease. FXS is caused by a loss of function mutation in the *FMR1* gene that encodes the fragile X mental retardation protein (FMRP). FMRP is needed for normal maturation and function of synapses. We have previously shown that the differentiation of embryonic cortical FXS neural progenitors is abnormal. We showed that human and mouse progenitors lacking FMRP generate more neurons with short neurites when compared to wild-type progenitors. Alterations of brain-derived neurotrophic factor (BDNF) and its TrkB receptor signaling contribute to the aberrances of FXS progenitor differentiation and the morphology of newborn neurons is normalized in progenitor cultures propagated from double transgenic *Fmr1*-KO mice (dMT) carrying a deletion in one copy of the *Bdnf* gene. The dMT mice deficient in both BDNF and FMRP show impaired contextual fear learning that is more pronounced than that with a deletion of only one of these genes. However, part of the FXS behavioural phenotype is normalized in the dMT mice.
We investigated the differentiation and migration of cortical progenitors propagated from embryonic brains of *Fmr1*-KO, and wild-type (WT) mice more detailed using an automated Cell-IQ® imaging system, intracellular calcium imaging, and immunocytochemistry. We observed an increased mobility of neurons differentiated from FMRP-deficient neurospheres when compared to WT controls as seen in neurospheres after exposure to BDNF. Alterations in the migration distance of doublecortin (DCX) and calbindin immunoreactive neurons were observed and the migration distance of outmost neurons lacking FMRP was increased. An increased expression of tissue plasminogen activator in astrocytes contributed to impaired cell migration in the absence of FMRP.
The results implicate BDNF in the migration defect of neurons in FXS and suggest a role for glia in the aberrant differentiation of FMRP-deficient neural progenitors.

T-3233

INDUCED EXPRESSION OF DUX4 DRIVES NEUROGENESIS IN MOUSE EMBRYONIC STEM CELLS.

Abhijit Dandapat, Lynn Hartweck, Michael Kyba
pediatrics, University of Minnesota, Minneapolis, MN, United States

Ectopic expression of the double homeodomain protein DUX4 is hypothesized to cause facioscapulohumeral muscular dystrophy (FSHD). Although future strategies are being explored to inhibit DUX4 activity in FSHD patients, little known about the normal function of this protein. DUX4 is expressed in human pluripotent cells and testis. To test the idea that DUX4 may be involved in initiating a germ lineage program, we tested the effect of DUX4 expression at different stages during *in vitro* differentiation of murine embryonic stem cells. We find that even low levels of DUX4 expression reduces pluripotency: pluripotency markers are downregulated and the cells rapidly differentiate even in the presence of LIF and BMP4. Microarray analysis showed that DUX4 induced a neurectodermal program rather than the expected germ lineaage program. We re-examined embroid body (EB) differentiation, with transient DUX4 expression. DUX4 severely inhibited mesodermal differentiation and the cells exhibited neurogenic potential. Even in serum-containing medium, DUX4 expression promoted neurogenesis, allowing the differentiation of TuJ1+ neurons compared with minimal neurogenic potential in controls. These data suggest that the involvement of DUX4 in neurogenesis should be examined in the testing of any DUX4 therapies.

T-3234

COMPARISON OF THE MULTIPOTENCY OF NESTIN-EXPRESSING STEM CELLS IN THE TONGUE FUNGIFORM PAPILLA AND VIBRISSA HAIR FOLLICLE

Sumiyuki Mii[1], Yasuyuki Amoh[2], Kensei Katsuoka[2], **Robert M. Hoffman**[1]
[1]AntiCancer Inc., San Diego, CA, United States, [2]Department of Dermatology, Kitasato University School of Medicine, Kanagawa, Japan

We have previously reported that hair follicles contain multipotent stem cells which express nestin and participate in follicle growth at anagen as well as in the extension of the follicle sensory nerve. The nestin-driven green fluorescent protein (ND-GFP) transgenic mouse labels all nestin-expressing cells with GFP. In vitro, the hair follicle nestin-GFP cells can differentiate into neurons, Schwann cells, and other cell types. In this study, we observed nestin-expressing cells which have similar characteristics in the fungiform papilla on the tongue. The nestin-expressing cells in the fungiform papilla are located around a peripheral sensory nerve immediately below the taste bud. The nestin-expressing cells of the fungiform papilla co-expressed the neural crest cell marker $p75^{NTR}$. The fungiform papilla also exhibited sphere formation in suspension culture in DMEM-F12 medium supplemented with basic fibroblast growth factor (bFGF). The spheres consisted of nestin-expressing cells that co-expressed $p75^{NTR}$ and which developed expression of the stem cell marker CD34. $P75^{NTR}$, CD34 and nestin expression indicated the cells comprising the fungiform papilla spheres were in an undifferentiated state. The nestin-expressing cells of these sphere could readily differentiate into β III tubulin-positive neurons, GFAP-positive glial cells, K15-positive keratinpcytes, and SMA-positive smooth muscle cells after transfer to RPMI medium 1640 with 10% fetal bovine serum (FBS). This nestin-expressing fungiform papilla cells and the nestin-expressing hair follicle cells have common features of cell morphology, expressed proteins, and the ability to differentiate to multiple cell types.

T-3235

INTEGRATION OF GENOME-WIDE APPROACHES IDENTIFIES LNCRNAS OF ADULT NEURAL STEM CELLS AND THEIR PROGENY IN VIVO

Alexander D. Ramos[1], Aaron Diaz[4], Abhinav Nellore[4], Ryan N. Delgado[1], Ki-Youb Park[1], Gabriel Gonzalez-Roybal[1], Michael C. Oldham[2], Jun S. Song[3], Daniel A. Lim[1]
[A]Department of Neurological Surgery, [B]Department of Neurology, [C]Institute for Human Genetics, [1]Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, UCSF, San Francisco, CA, United States, [2]Institute for Human Genetics, UCSF, San Francisco, CA, United States

Long noncoding RNAs (lncRNAs) have been described in cell lines and various whole tissues, but lncRNA analysis of development in vivo is limited. The subventricular zone (SVZ) of the adult mouse brain represents an ideal system for the study of lncRNAs in vivo. Throughout life, SVZ neural stem cells (SVZ-NSCs) generate large numbers of neuroblasts that migrate to the olfactory bulb (OB) where they differentiate into interneurons. We leveraged this unique developmental system to interrogate lncRNA expression and function. Here, we comprehensively analyze lncRNA expression for the adult mouse SVZ NSC lineage and assess function of select lncRNAs with shRNA-mediated knockdown. We utilize complementary genome-wide techniques including RNA-seq, RNA CaptureSeq, and ChIP-seq to associate specific lncRNAs with neural cell types, developmental processes, and human disease states. By integrating data from chromatin state maps, custom microarrays, and FACS purification of the SVZ lineage, we stringently identify lncRNAs with potential roles in adult neurogenesis. shRNA-mediated knockdown of two such lncRNAs, Six3os and Dlx1as, indicate roles for lncRNAs in the glial-neuronal lineage specification of multipotent adult stem cells. Six3os knockdown reduced the production of both Tuj1+ and OLIG2+ cells, indicating a role for Six3os in specifying both neuronal and oligodendroglial lineages. This was accompanied an increase in GFAP+ cells after differentiation, suggesting that this lncRNA is required for lineage commitment from the GFAP+ NSCs of the SVZ. In contrast, Dlx1as knockdown selectively reduced production of Tuj1+ neuroblasts and not cells of the oligodendrocyte lineage. Dlx1as is robustly expressed during early differentiation, this induction of expression coincides with the enrichment of H3K27 demethylase JMJD3 at the Dlx1as transcriptional start site and loss of the H3K27me3 repressive histone mark, suggesting that chromatin modifying factors can regulate lncRNA expression. Furthermore, we demonstrate that knockdown of Dlx1as selectively inhibits the expression of Dlx family members, including its neighbors Dlx1 and Dlx2, indicating that lncRNAs may be required as part of the neurogenic transcriptome. Taken together, our genome-wide analysis and workflow thus provide a uniquely coherent in vivo lncRNA analysis and demonstrates key roles for lncRNAs in an adult neural stem cell population.

T-3236

LOW PROTEIN MATERNAL DIET AFFECTS NEURAL STEM CELLS IN THE FOETAL MOUSE BRAIN.

Phoebe J. Smith[1], Stephanie J. Marfy-Smith[2], Tom P. Fleming[2], **Sandrine Willaime-Morawek**[1]
[A]Faculty of Medicine, [B]Centre for Biological Sciences, [1]University of Southampton, Southampton, United Kingdom

In the modern world, despite the latest science and technology,
malnutrition is widespread and the World Health Organisation states it as the greatest single threat to the world's public health. With this is mind many studies have tried to investigate the consequences of inadequate nutrition on human development and health. This study focuses specifically on the effect of a maternal low protein diet (LPD) on the development of the foetal brain.
Whilst previous studies indicate LPD to have a negative effect on foetal
brain development, we have investigated specifically whether maternal LPD negatively affects neural stem cell derivation and neurogenesis in the foetal mouse brain.
MFI female mice were fed three different diets from conception: normal
protein diet (NPD, 18% casein), low protein diet (LPD, 9% casein) or an
embryonic LPD (Emb-LPD: LPD from E0- 3.5, NPD thereafter). Brains from E14.5 mouse foetuses were collected and ganglionic eminence neural stem cell functions and properties were investigated using neurosphere culture and staining techniques.
The number of primary neurospheres was decreased in LPD vs NPD ($p<0.05$). When analysed by size groups, the overall difference in primary spheres across diets is accounted for by the small spheres (100-200µm), whereas no difference is seen in the large spheres (>400µm). No overall difference in the formation of secondary neurospheres was detected between treatments following bulk passaging of the primary spheres. However, when passaging small primary spheres separately from large primary spheres, the number of secondary neurospheres formed was affected by maternal diet ($p<0.05$). We have also shown that the difference between the diets can be observed as early as day two of the neurosphere assay. These neurosphere cultures suggest that the LPD, and to a lesser degree the Emb-LPD, may decrease the self-renewal capacity of the neural stem cells, and the smaller spheres, but not the larger spheres, retain this limitation into the second generation of neurospheres.

Moreover, Emb-LPD seems to promote a higher neurogenesis rate in the ganglionic eminences *in vivo*, revealed by immunohistochemistry for Nestin, a stem/progenitor marker and Beta-III-tubulin, an early neuronal marker. Overall, our work may reveal a profound effect of maternal protein diet on neural stem cell self-renewal and neurogenesis in the foetus and may also point to the importance of the timing and duration of the protein restriction.
Work funded by a BBSRC grant BB/I001840/1 to TPF.

T-3237

EFFECTS OF ADULT NEURAL PRECURSOR-DERIVED MYELINATION ON AXONAL FUNCTION

Crystal A. Ruff[1], Hui Ye[3], Jean M. Legasto[1], Natasha A. Stribbell[1], Jian Wang[1], Liang Zhang[2], Michael G. Fehlings[1]
[A]Medicine, [B]Fundamental Neurobiology, [1]University Health Network, Toronto, ON, Canada, [2]Biology, Loyola University, Chicago, IL, United States

Stem cell repair shows substantial translational potential for neurological repair, but its mechanisms of action remain unclear. This study aimed to investigate whether transplanted stem cells can induce comprehensive functional remyelination. In this study, sub-ventricular zone (SVZ)-derived adult neural precursor cells (aNPCs) were injected bilaterally into major cerebral white matter tracts of myelin-deficient shiverer mice on P0, P7 and P21. Tri-potential NPCs, when transplanted in vivo, integrated anatomically and functionally into local white matter and preferentially became Olig2+, MAG+, MBP+ oligodendrocytes, rather than GFAP+ astrocytes or NF200+ neurons. Processes interacted with axons and TEM showed multilamellar axonal ensheathment. Nodal architecture was restored and by quantifying these anatomical parameters a computer model was generated that accurately predicted action potential velocity, determined by ex vivo slice recordings. Myelinated axons exhibited faster conduction, lower activation threshold, less refractoriness and improved response to high frequency stimulation than dysmyelinated counterparts. Furthermore, they showed improved resilience to ischemic insult, a promising finding in the context of perinatal brain injury. This study describes, for the first time mehanistically, the functional characteristics and anatomical integration of non-immortalized donor-SVZ-derived murine aNPCs in the dysmyelinated brain at key developmental timepoints.

T-3238

PRE-CLINICAL STUDIES OF HUMAN PARTHENOGENETIC STEM CELL BASED THERAPY FOR THE TREATMENT OF PARKINSON'S DISEASE

Ruslan A. Semechkin[1], Rodolfo Gonzalez[1], Ibon Garitaonandia[1], Alina Ostrowska[1], Francesca S. Boscolo[2], Larisa Agapova[1], Tatiana Abramihina[1], Gerald Wambua[1], Alexander Noskov[1], Louise C. Laurent[4], D. Eugene Redmond, Jr[5], Evan Y. Snyder[3]
[1]International Stem Cell Corporation, Carlsbad, CA, United States, [A]Muscle Development and Regeneration Program, [B]Stem Cells and Regenerative Biology Program, [2]Sanford-Burnham Medical Research Institute, La Jolla, CA, United States, [3]Department of Reproductive Medicine, UC San Diego, La Jolla, CA, United States, [4]Departments of Psychiatry and Neurosurgery, Yale University School of Medicine, New Haven, CT, United States

Parkinson's disease (PD) is a neurodegenerative disorder caused by a progressive degeneration of midbrain dopamine neurons in the substantianigra pars compacta. There is currently no cure for PD and treatments such as deep brain stimulation andlevodopacan alleviate some of the symptoms, but lose efficacy over time. The localized nature of the loss of DA neurons in the substantianigra offers the opportunity for cell replacement therapy in PD patients.Human parthenogetic stem cells (hpSCs) are a good source for cell replacement therapies because they can divide indefinitely and provide an unlimited source of homogeneous cell populations homozygous at the human leukocyte antigen loci, significantly reducing immunogenicity and simplifying immune matching in clinical applica-

tions. Here we report the results of pre-clinical in vivo efficacy studies of hpSC based therapies for the treatment of PD.We generated through chemically defined methods highly pure and homogeneous populations of neuronal cells from hpSCs and transplanted them into rodent and non-human primate models of PD. In the rodent model, we used 6-OHDA-induced Parkinson's disease rats,a well-established and validated model of PD with pharmacological induction of rotational behavior to assess the effects of lesions and potential of cell therapy. We observed long term survival, engraftment, dopamine release, and improvement of amphetamine-induced behavior over sham control. In the non-human primate model, we used African green monkeys with MPTP-induced Parkinson's disease, which is the most complete and identical model of human Parkinson's disease that has been studied of any species.In this study we used asymptomatic monkeys, which have reduced dopamine levels in the nigrostriatal pathway, but no functional impairments. Parkinson's scores were recorded by blinded observers twice per day, intake of food and water was monitored daily and body weight was recorded weekly. We observed survival, engraftment, dopamine release, stable behavioral scores and no adverse effects.These results prove the in vivo efficacy of human parthenogenetic stem cell derived products and bring them a step closer to their potential clinical application in Parkinson's disease.

T-3241

FUNCTIONAL CHARACTERISATION OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED NEURONS

Tina C. Stummann[1], Janne Jensen[1], Anders C. Lassen[1], Kristen Frederiksen[2], Karina Fog[1], Jan Egebjerg[1]
[A]Neurodegeneration, [B]Synaptic Transmission, [1]H. Lundbeck A/S, Valby, Denmark

Neurons derived from human iPS cells have potential to improve *in vitro* models for neurodegenerative diseases, but a clear understanding of the neuronal functional matureness of the differentiated cultures is crucial for such purposes. In this study, iPS-derived neuronal cells from healthy individuals where characterised for marker expression and functional properties. The neuronal derivatives exhibited clear neuronal morphology with neurite outgrowth after 4-5 weeks of differentiation. They continued to be proliferative for at least 100 days and were successfully passaged several times. The proliferation was much lower than for undifferentiated iPS cells. Real-time PCR showed up-regulation of several neuronal marker genes and immunohistochemistry demonstrated the appearance of Neurofilament H, β-Tubulin III, Microtubule-associated protein 2 (MAP2) and Microtubule-associated protein tau (MAPT) positive cells. A fluorescent kinetic plate reader was used to determine intercellular calcium changes in cells. The results were persistent between different passages of derivatives possessing neuronal morphology. Depolarisation of the membrane potential by increasing the extracellular potassium levels caused an increase in the cytosolic calcium levels. Stimulation with glutamate combined with glycine in the absence of extracellular magnesium resulted in a considerable increase in cytosolic calcium. A substantial amount of this could be blocked by the NMDA receptor blocker MK801. In line, addition of NMDA also increased the cytosolic calcium levels. These data indicate the presence of glutamate receptors such as the NMDA receptors. Nifedipine reduced the glutamate/glycine induced rise in intracellular calcium pointing to the presence of voltage-gated calcium channels. Patch clamp recordings in whole-cell voltage-clamp mode showed the presence of voltage-gated sodium and potassium currents in some but not all cells. In conclusion, the continuous proliferative capacity of the neuronal differentiated iPS cells implies that the neurons may not be completely maturated. However, the derivatives did indeed exhibited neuronal marker expression and functional characteristics reflecting those of neuronal cells

T-3242

HEPARAN SULFATE-DEPENDENT GROWTH FACTOR SIGNALING IN NEURAL DIFFERENTIATION OF EMBRYONIC STEM CELLS

Anqi Xiong[1], Soumi Kundu[1], Tanja Paavilainen[1], Maud Forsberg[2], Jinping Li[2], Karin Forsberg-Nilsson[1]
[A]Dept. of Immunology, genetics and pathology, [B]Dept. of Medical biochemistry and micobiology, [1]Uppsala University, Uppsala, Sweden

Heparan sulfate (HS) proteglycans, present on cell surfaces and in the extracellular matrix, interact with growth factors and morphogens to influence growth and differentiation of cells. The NDST (glucosaminyl N-deacetylase/ N-sulfotransferase) enzymes have a key role during HS biosynthesis, greatly influencing total sulfation of the HS chains, and the lack of these enzymes results in early embryonic lethality. Our previous study shows that embryonic stem cells lacking both NDST1 and NDST2, expressing a very low sulfated HS, can only take initial steps towards neural differentiation and then become arrested in a primitive ectoderm stage. HS mediated signaling is thus an early and necessary step in the commitment to the neural lineage.
The cleavage of HS side chains is mediated by the enzyme heparanase (Hpse), an endo-β-D-glucuronidase that releases bioactive molecules thereby affecting proliferation and differentiation. To further understand the regulation of neural differentiation via HS, we studied the role of heparanase. For this purpose, neural stem/progenitor cells (NSPCs) were isolated from embryonic brain of heparanase knockout (Hpse-KO) and wild type mice. NSPCs from both genotypes were capable of forming neurospheres and subsequently differentiating to neuron and glia, in accordance with the mild phenotype of the Hpse-KO mice.
Next, ES cell lines were generated from blastocysts of Hpse-KO mice, and mice overexpressing Hpse (Hpse-TG). The ES cells were confirmed for the expression of pluripotency markers and a normal karyotype. When injected into immuno-deficient mice, they formed teratomas, with Hpse-TG ES cells giving larger tumors than Hpse-KO and wt ES cells. Using monolayer neural differentiation we found that ES cells from both genotypes could be differentiated into NSPC. However, the Hpse-TG ES cells had a proliferation rate during neural induction, which was several times higher than wt or Hpse-KO ES cells, showing that HS sulfation affects progenitor proliferation during the generation of neural stem cells from pluripotent ES cells.
NSPCs from both Hpse-TG and Hpse-KO ES cells were able to generate neurons and astrocytes upon differentiation induced by mitogen withdrawal. Unexpectedly, Hpse-TG cultures had a high amount of oligodendrocytes (15%). In accordance with previous publications, wt ES cells only rarely generated oligodendrocytes by this protocol. We therefore measured Hpse levels during wt neural differentiation and found that Hpse expression declined during formation of neurons and glia of wt cels. Thus, a continuous high level of Hpse in the HPSE-tg NSPC coincides with the observed shift in differentiation potential. We are currently investigating how altered heparanase levels affect signaling pathways known to affect oligodendrocyte differentiation. Our hypothesis is that Hpse either promotes oligodendrocyte differentiation or alternatively, releases a block that normally inhibits oligodendrocyte formation.

T-3243

BAC MEDIATED FLUORESCENT LABELING OF HESCS AND PROGENY FOR SIMPLE AND EFFECTIVE CELL TRACKING

Anders Smith, **Arturo Zapata**, Sherry Zhang, Binhai Zheng
University of California, San Diego, San Diego, CA, United States

Human embryonic stem cells (hESCs) offer unprecedented potential for both science and medicine. The pluripotent nature of these cells makes them ideal tools for developmental research. Because they are non-transformed human cells, they may also be especially valuable for the development of new pharmaceutical agents and high throughput screens. Likewise, hESCs serve as excellent starting points for developing cell transplant and cell replacement therapies, particularly for tissue types that exhibit poor endogenous regenerative capabilities such as the human central nervous system. Despite their great promise, there remain some obstacles that must be overcome before hESCs can be utilized to their full potential. One obstacle that is frequently encountered is the need of a means to effectively track and identify hESCs and their progeny, especially in vivo following transplant. While there exist some hESC lines that have been modified to express persistent fluorescence, restricting research to only these lines would greatly limit the number of available options. Here we strive to develop a non-viral, genetic tool, which can be incorporated with relative ease into virtually any hESC or iPS line resulting in bright, persistent, clonally stable fluorescence. Through the implementation of recombineering techniques and bacterial artificial chromosome (BAC) transgenesis, we have generated a series of BAC constructs that utilize the murine ROSA26 locus to drive widespread fluorescent protein expression. Our initial construct utilized no additional promoter or enhancer regions aside from the ROSA26 locus. Once electroporated into the HUES9 hESC line, this BAC effectively labeled the cells with ubiquitous, clonally stable fluorescence. What's more, fluorescence persisted throughout neuron-

al differentiation, demonstrating the BAC's effectiveness as a marker for both stem cells and their differentiated progeny. Seeking to further enhance the intensity of fluorescence, we generated several additional BACs, by incorporating CAG or PGK promoters in conjunction with WPRE enhancers and different fluorescent proteins, into the ROSA26 BAC construct. Early observations indicate that these modifications have been successful in noticeably enhancing the fluorescence of the transduced hESCs. Meanwhile, fluorescent reporter gene expression from stronger promoters may be subjected to silencing effects. We are currently investigating whether this effect is promoter-specific. BAC transgenesis represents a viable alternative to other genetic manipulations for effective labeling and tracking of embryonic stem cells and their progeny.

T-3244

SMELL YOU LATER: ACTIVATION OF RESERVE STEM CELLS IN THE OLFACTORY EPITHELIUM

Nikolai Schnittke, Daniel Herrick, James E. Schwob
Anatomy and Cellular Biology, Tufts Univ Sch of Medicine, Boston, MA, United States

As a neural system the olfactory epithelium has a remarkable capacity to repair itself after injury; the population of sensory neurons is restored (including a near-perfect restoration of the patterns of odorant receptor expression), and the olfactory bulb is reinnervated to restore function. Key to that capacity is the persistence of two kinds of neurocompetent stem cells throughout life: globose basal cells (GBCs) and horizontal basal cells (HBCs). The latter basal cell type appears to be a quiescent or reserve population of stem cells - they resemble the basal cells of respiratory epithelium closely, except they are late to emerge, take developmental origin from GBCs or GBC-like cells, divide rarely under normal conditions, and contribute to epithelial renewal only when the epithelium has been severely damaged (loss of neurons is not sufficient to raise them out of their dormancy). We have been analyzing the process by which HBCs are stimulated to contribute via a CFU, orthotopic transplantation assay, and by studying their capacity to contribute to the epithelium when left in situ. We have demonstrated that down-regulation of the transcription factor p63 is both necessary and sufficient to accomplish activation by using retroviral transduction to overexpress p63, which drives cells to become or remain HBCs, and conditional recombination to eliminate p63 and activate TdTomato expression. When HBCs are activated by knockout of p63, the GBCs that they generate remain active as stem and progenitors for many months either in the context of the normal or injured-recovered epithelium. Nonetheless, HBCs must be activated in situ in order to function as multipotent progenitors after transplantation into the lesioned epithelium. When harvested from normal epithelium the HBCs seldom engraft and very rarely give rise to neurons, in contrast to the robust capacity of GBCs from normal epithelium to make all cell types when subject to similar challenge. If HBCs are isolated at a time point after injury when p63 levels reach a nadir, then they do give rise to all of the cell types of the epithelium as they would if left in place. Interestingly, when GBCs are completely destroyed, the HBCs seem to be only capable of producing respiratory epithelium. Finally, Notch activation may be a key component of activation; overexpression of NICD following conditional recombination is sufficient for a low level of activation within the normal epithelium. Thus, the regulation of this reserve population is complex, but may provide an opportunity for therapeutic repair in aging populations where the epithelium has apparently undergone neurogenic exhaustion.

T-3245

EXPANDABLE OLIG2-POSITIVE HUMAN NEURAL STEM CELLS CAN BE SURROGATE OF HUMAN OLIGODENDROCYTE PROGENITOR CELLS

Tsuneo Kido
Stem Cell Medicine LLC, Germantown, MD, United States

Oligodendrocytes are known to play a critical role in the pathogenesis of many neurological disorders including spinal cord injury, multiple sclerosis, schizophrenia, and congenital demyelinating diseases. A re-myelination strategy for spinal cord injury is well established and several treatments using neural stem cell/oligodendrocyte progenitor cell have undergone preclinical studies and/or clinical trials [i.e. human embryonic stem cell (HESC)-

derived human oligodendrocyte progenitor cells (hOPCs) (Geron Corp., terminated Phase I trial), human neural stem cells (hNSCs) (Stem Cells, Inc., Phase I clinical trial in Switzerland) (Neuralstem, Inc.), autologous schwann cell (Miami project) and autologous bone marrow-derived cell (SanBio, Inc.)]. However, there are pros and cons for these cells in term of efficiency. hNSCs seem to have higher survival rate than other cells but other cells have higher differentiation potency into myelin-forming cells.
hNSCs are usually cultured in a floating condition, called neurosphere culture, to maintain their multi-potential and proliferation potential. However, neurospheres contain many different progenitors and differentiated cells and the ratio of neural stem cells is usually very low. Recently, hNSCs could be expanded under adherent monolayer culture condition on laminin-coated cultureware in the presence of EGF and bFGF. However, their differentiation potential into oligodendrocyte seems to be low. I developed a new culture method to expand hNSCs >10,000,000 times on adherent culture condition and presented at ISSCR 10th annual meeting in Yokohama. These expanded cells displayed a homogeneous morphology and most of them expressed neural stem cell markers like CD133 (100% by flow cytometry), Sox2, and nestin (passage 13). When they were differentiated in a serum-containing medium, neuron (ßIII-Tublin positive , Neurofilament L positive and/or MAP-2 positive), astrocytes (GFAP positive), and oligodendrocytes (O4 positive, GalC positive and/or MBP positive) could be observed. These data indicated that they retained multipotency even after expansion and that they were neural stem cells.
Interestingly, they expressed olig2 and differentiated into pro-oligodendroglia (phase bright multipolar cell; O4 positive/GalC negative) and immature oligodendrocytes (phase bright multipolar cell with spider web structure; O4 positive/GalC positive/MBP negative) spontaneously even in a proliferation condition (1-5% of total cells). When they were differentiated by growth factor withdrawal and cAMP induction, 99% of total cells became O2A progenitor cells (bipolar or multipolar cell; A2B5 positive/O4 weakly positive/NG2 positive/ GD3 positive) or pro-oligodendrocytes (multipolar cell; O4 positive/GalC negative) after 4 days. In a long-term differentiation condition (1 week), many immature oligodendrocytes (O4 positive/GalC positive/MBP negative) and mature oligodendrocytes (O4 positive/GalC positive/MBP positive) could be observed.
They seem to have advantage of both hNSCs and oligodendrocyte progenitor cells and can be used for cell therapies. We are testing their myelination potency in shiverer mice and going to test their efficacy in spinal cord injury model mice and Pelizaeus-Merzbacher disease model mice.

T-3246

MITOCHONDRIAL P53-DEPENDENT REGULATION OF MITOCHONDRIAL DAMAGE AND NEURAL STEM CELL FATE

Susana Solá[1], Joana Xavier[2], Ana Luísa Morgado[1], Cecília MP Rodrigues[1]
[A]Department of Biochemistry and Human Biology, [B]Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), [1]Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

We have previously shown that apoptosis-regulatory proteins, including p53, play a pivotal role in neural differentiation, through mechanisms independent of cell death. Recently, p53 is also emerging as an important regulator of mitochondrial survival response, as it mediates mtDNA integrity and oxidative protection. Further, reactive oxygen species (ROS) function as signaling molecules of autophagy, a mechanism that results in lysosomal degradation of cytoplasmic constituents critical to prevent cell death. Our results showed that mitochondrial apoptotic events occur at early stages of mouse neural stem cell (NSC) differentiation, such as increase of ROS production, mitochondrial membrane depolarization and cytochrome c release, with no evidence of apoptosis. Curiously, throughout neural differentiation, mitochondrial mass decreased, while the lipidated LC3-II marker of autophagy increased. In addition, p53 was actively translocated to mitochondria. To further understand the precise role of p53 in mitochondrial events, we modulated p53 mitochondrial translocation in NSCs undergoing differentiation by transfecting cells with either wild-type RECQL4, an helicase essential for the transport of p53 to the mitochondria, RECQL4-Δ84, which cannot translocate to the mitochondria, and RECQL4-GST, unable to physically interact with p53. Higher levels of mitochondrial p53 were observed in RECQL4-overexpressing cells when compared with control (mock), while ROS production and mitochondrial membrane depolarization were markedly reduced. Importantly, p53-mediated mitochondrial alterations were not seen in NSC transfected with either RECQL4-Δ84 or RECQL4-GST overexpression

plasmids. Finally, activation of autophagy was correlated with ROS production, in association with p53 cellular relocation. In conclusion, our results revealed that both mitochondrial translocation of p53 and autophagy are responsible for the resistance mechanisms by which cells survive to mitochondrial apoptosis-associated events during neural differentiation.

T-3247

HUMAN ORGANOTYPIC SLICE CULTURE AS A TOOL TO STUDY ALLOGENEIC NEURAL CELL THERAPY IN SPINAL CORD INJURY

Cinzia Calzarossa[1], Jia Liu[1], Lena Holmberg[1], Katja Puttonen[2], Jari Koistinaho[2], Elisabet Åkesson[1]
[1]Dept of Neurobiology, Care Sciences and Society, Karolinska Institutet, Stockholm, Sweden, [2]A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland

Stem cells are a promising source in the study and development of neural cell therapy, including a therapy for spinal cord injury (SCI). However, so far, we lack a model to study allogeneic human neural cell therapy to evaluate the interaction of human donor and host neural tissue. We have developed a novel system to evaluate human neural progenitor cells (hNPCs) derived from induced pluripotent stem cells and fetal derived neural cells in a human organotypic slice culture. This model offers the possibility to analyze the interaction between human donor and host spinal cord cells in an allogeneic setting. Slices from human first trimester fetal spinal cord, were cultured in the interface between air and medium up to 21 days. To induce spinal cord contusion injuries, slices were lesioned using the Infinitive Horizon spinal cord impactor, with a force of 25 kdyne. After 1h, hNPCs were grafted or sham injections were performed and 5 days later slices with and without lesions and grafts were analyzed by immunohistochemistry and flow cytometry The human spinal cord slices presented relatively stable features 7 to 21 days in unlesioned cultures based on markers for cell proliferation (ki67), progenitor cells (nestin), neurons (microtubule-associated protein 2, MAP-2), astrocytes (glial fibrillary acidic protein, GFAP) and microglia (Iba 1 and HLA-DR+/CD11b+/CD45low). However, after SCI and neural cell therapy changes were observed in MAP2, GFAP, Iba1 appearance and distribution. Particularly, we saw increased activation of Iba1 and GFAP positive cells, in response to injury but also to hNPC grafts. These findings are at least partly in contrast to previous observations after human neural xenografts into the adult injured spinal cord.
We conclude that this novel model of human allogeneic neural cell therapy in slice cultures represent an important platform to study host donor interactions, in the evaluation and development of neural cell therapies.

T-3248

SAFETY AND ENGRAFTMENT ASSESSMENTS OF INTACT PARENCHYMA VERSUS EPICENTER AS A TRANSPLANTATION SITE FOR HUMAN NEURAL STEM CELLS FOR SPINAL CORD INJURY THERAPY

Katja M. Piltti[1], Desiree L. Salazar[4], Nobuko Uchida[5], Brian J. Cummings[2], Aileen J. Anderson[3]
[A]Institute for Memory Impairments & Neurological Disorders, [B]Sue & Bill Gross Stem Cell Research Center, [C]Anatomy & Neurobiology, [1]University of California Irvine, Irvine, CA, United States, [2]Ludwig Institute for Cancer Research, University of California San Diego, La Jolla, CA, United States, [3]StemCells Inc., Newark, CA, United States

Neural stem cell transplantation may have the potential to yield repair and recovery of function in central nervous system (CNS) injury and disease, including spinal cord injury (SCI). Multiple pathological processes are initiated at the epicenter of a traumatic spinal cord injury; these are generally thought to make the epicenter a particularly hostile microenvironment. Conversely, the injury epicenter is an appealing potential site of therapeutic hCNS-SCns transplantation because of both its surgical accessibility and the avoidance of spared spinal cord tissue. In this study, we compared transplantation into the spinal cord injury (SCI) epicenter (EPI) versus intact rostral/caudal parenchyma (R/C) in contusion-injured athymic nude (ATN) rats, and assessed the survival, differentiation, and migration of human central nervous system-derived neural stem cells (hCNS-SCns). Regardless of transplantation site, hCNS-SCns survived and proliferated; however, the total number of hCNS-SCns quantified in the R/C transplant an-

imals was twice that in the EPI animals, demonstrating increased overall engraftment. Migration and fate profile were unaffected by transplantation site. However, although transplantation site did not alter the proportion of human astrocytes, EPI transplantation shifted the localization of these cells, and exhibited a correlation with CGRP fiber sprouting. Critically, no changes in mechanical allodynia or thermal hyperalgesia were observed. Taken together, these data suggest that the intact parenchyma may be a more favorable transplantation site for hCNS-SCns than the injury epicenter in the sub-acute period post SCI.

T-3251

SPINAL MOTOR NEURONS GENERATED FROM INDUCED PLURIPOTENT STEM CELLS DERIVED FROM SPINAL MUSCULAR ATROPHY PATIENTS FAILED TO CLUSTER ACETYLCHOLINE RECEPTORS AT THE NEUROMUSCULAR JUNCTIONS

michiko yoshida, shiho kitaoka, mayu yamane, kayoko tsukita, haruhisa inoue, megumu saito, tatsutoshi nakahata
center for iPS cell research and application, kyoto, Japan

Objective: To establish an in vitro model of neuromuscular junction (NMJ) connectivity using motor neurons differentiated from spinal muscular atrophy (SMA) patient-derived induced pluripotent stem cells (iPSCs) in order to evaluate the major factors contributing to the pathogenesis of SMA.
Background: SMA is a neuromuscular disorder caused by mutations of the survival of motor neuron 1 (SMN1) gene. It remains unclear why the reduction of the ubiquitously expressed SMN protein selectively affects the neuromuscular system in SMA patients. Recent findings suggest that impaired NMJ formation is an important hallmark in SMA. However, the contribution of NMJ pathology to the pathogenesis of SMA remains unclear due to the limited availability of tissue samples.
Design/Methods: We established human iPSCs from SMA patients and healthy control subjects. The iPSCs were differentiated into motor neurons, and were co-cultured with mouse myotubes to form NMJ *in vitro*. The number of motor neurons and the mean area of acetylcholine receptor(AChR) clustering were evaluated by immunocytochemistry.
Results: While AChR clustering was successfully established on myotubes co-cultured with control iPSC-derived motor neurons, this was significantly impaired in those cultured with SMA iPSC-derived motor neurons. On the other hand, no significant neuronal death was observed in the SMA iPSC derived motor neurons compared to those from healthy controls.
Conclusions: We successfully developed an *in vitro* model of NMJ, and by using SMA-iPSCs, we demonstrated that low levels of SMN in the motor neurons are responsible for the impairment of AChR clustering. Our findings imply that the loss of neuromuscular connections appears prior to motor neuronal death, thus suggesting that the NMJ defect is likely to be a major contributing factor to the pathogenesis of SMA. The current *in vitro* NMJ model is useful to dissect the pathophysiological mechanisms underlying the development of SMA, and to evaluate the efficacy of new therapeutic approaches.

T-3252

SYNAPTIC INHIBITION BY GABAERGIC PROGENITOR GRAFTS MEDIATES LONG-TERM SEIZURE SUPPRESSION IN MICE WITH TEMPORAL LOBE EPILEPSY

Katharine Henderson[1], Jyoti Gupta[1], Stephanie Tagliatela[2], Sara Royston[3], Nicholas Woods[1], Ethan Grund[1], Meghan van Zandt[1], Yuchio Yanagawa[4], Gloster B. Aaron[1], Janice R. Naegele[1]
[1]Department of Biology, Wesleyan University, Middletown, CT, United States, [2]Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA, United States, [3]Neuroscience Program and Medical Scholars Program, University of Illinois Urbana-Champaign, Urbana, IL, United States, [4]Department of Genetic and Behavioral Neuroscience, Gunma University Graduate School of Medicine, Maebashi, Japan

Acquired temporal lobe epilepsy (TLE) is associated with severe seizures and neuropathological changes including GABAergic interneuron loss, mossy fiber sprouting, and the development of recurrent pharmacoresistant seizures. Prior research in mice suggested that transplanting fetal brain-derived GABAergic progenitors into the brain could partially suppress seizures, however the cellular mechanisms remain poorly characterized. To identify mechanisms for seizure suppression, we harvested GABAergic progenitors from the medial ganglionic eminences (MGE) of embryonic day 13.5 mice expressing Venus under control of the vesicular GABA transporter (VGAT-Venus) and bilaterally injected either the cells plus media or media alone (controls) into the dentate gyrus after pilocarpine induction of TLE. Intracranial EEGs for periods of 100-140 days were used to evaluate seizure incidence. MGE grafts led to both increased expression of somatostatin or pavalbumin interneurons (Zheng et al. STEMConn 2013), indicative of an inhibitory fate, and also significantly fewer seizures (51% fewer seizures than TLE controls). A 6-8 week delay from transplantation until seizure suppression suggested a role for inhibitory synaptogenesis. Immunostaining confirmed that VGAT-Venus+ interneurons established extensive gephyrin-positive synapses with host brain granule cells. Whole-cell electrophysiological recordings of postsynaptic currents (PSCs) from granule cells near the MGE grafts revealed significantly increased ($p<0.01$) ratios of inhibitory (IPSCs) to excitatory postsynaptic currents (EPSCs). MGE grafts also reduced neuropathological changes in TLE mice, including mossy fiber sprouting and astrogliosis. Taken together, our results show that embryo-derived GABAergic interneuron progenitor grafts establish robust inhibitory synaptic connections and exert long-term seizure suppression in mice with chronic epilepsy.

T-3253

A STEM-CELL-BASED PHENOTYPIC ASSAY IDENTIFIES COMPOUNDS THAT PROTECT HUMAN NEURONS FROM DEGENERATION

Susanne Höing[1], York Rudhard[2], Peter Reinhardt[1], Michael Glatza[1], Mark Slack[2], Hans R. Schöler[1], Jared Sterneckert[1]

[1]*Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, Münster, Germany,* [2]*Evotec AG, Hamburg, Germany*

A Stem-Cell-Based Phenotypic Assay Identifies
Compounds that Protect Human Neurons from Degeneration

Susanne Höing[1], York Rudhard[2], Peter Reinhardt[1], Michael Glatza[1], Mark Slack[2], Hans R. Schöler[1,3], and Jared Sterneckert[1]

[1]Department of Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, Röntgenstrasse 20, 48149 Münster, Germany

[2]Evotec AG, Manfred Eigen Campus, Essener Bogen 7, 22419 Hamburg, Germany

[3]University of Münster, Medical Faculty, Domagkstrasse 3, 48149 Münster, Germany

Because the population of the world is living longer than before, the prevalence of neurodegenerative disorders, such as amyotrophic lateral sclerosis (ALS) and Parkinson's disease, is expected to increase. However, the treatments currently available for these disorders are generally ineffective. Consequently, better therapeutics are urgently needed. Aberrant activation of microglial cells, the resident immune cells of the central nervous system, plays an important role in the pathogenesis of neurodegenerative diseases including ALS. We generated an *in vitro* model of microglial-induced neurodegeneration using stem cells. Because stem cells are able to self-renew and differentiate, they can produce theoretically limitless numbers of functional neural cells. As a result, our model is scalable and can potentially be used in high-throughput screening (HTS) campaigns. To validate our model in preparation for HTS, we performed a pilot screen of more than 10,000 small molecules. We identified 12 hit compounds, which acted through diverse mechanisms including the inhibition of nitric oxide production by microglia, activation of the Nrf2 pathway in microglia and astrocytes, and direct neuroprotection. We confirmed that one class of compounds directly protected human neurons from degeneration in response to nitric oxide. In addition, we validated that NRF2 activation in human midbrain dopaminergic neurons protected them from Parkinsonian neurodegenera-

tion. These results indicate that our hit compounds could be ideal starting points for the development of new drugs to treat various neurodegenerative and neurological diseases.

T-3254

TRANSPLANTATION OF PRE-DIFFERENTIATED HUMAN NEURAL STEM CELLS REPOPULATE AND FORM NEW SYNAPTIC CONTACTS WITHIN THE STROKE-DAMAGED BRAIN

Hima C. Abeysinghe[1], Laita Bokhari[1], Jeremy M. Crook[2], Gregory J. Dusting[3], Carli L. Roulston[1], Nao R. Kobayashi[2]
[1]O'Brien Institute, Melbourne, Australia, [A]Centre for Neural Engineering, [B]Centre for Eye Research Australia, [2]The University of Melbourne, Melbourne, Australia

Currently there is no effective treatment for stroke sufferers beyond the hyperacute phase (4.5 hours). The use of stem cell based therapies to promote brain repair offers new hope for improving functional restoration after stroke. In the current study, we have isolated and characterized human neural stem cells (hNSCs) from the ventricular zone (VZ) and directed their differentiation into neural cells including GABAergic neurons. We investigated the effect of pre-differentiated VZ-hNSC transplants into the rat brain 7 days after endothelin-1 induced stroke. Undifferentiated, pre-differentiated VZ-hNSCs or vehicle were stereotaxically injected into the rat brain (n=10/group) at 8 predetermined sites to target both the striatum and cortex. Graft cell survival, differentiation status and neurite outgrowth were assessed 28 days post-transplantation using immunohistochemistry and confocal microscopy. Pre-differentiated VZ-hNSCs maintained their neuronal phenotype as evidenced by human nuclear antigen (hNA) co-labeled with β-III-tubulin, GABA and GAD. In addition, pre-differentiated stem cells upregulated pre-synaptic marker synaptophysin suggesting formation of new synaptic contacts within the graft. Finally, pre-differentiated grafts located in the border region of the infarct exhibited longer processes compared to those within the core. Undifferentiated VZ-hNSCs were either double positive for hNA and GFAP or remained undifferentiated by expressing Nestin and Ki67. Our findings suggest pre-differentiating VZ-hNSCs into neuronal cells prior to transplantation promotes neuron repopulation within the stroke-damaged brain with synaptogenesis indicating integration and further maturation. Our work represents a novel approach to the therapeutic application of hNSCs and restoration of the stroke affected brain.

T-3255

IMPROVEMENT OF STROKE-DAMAGED HOST ENVIRONMENT BY HUMAN NEURAL STEM CELLS OVEREXPRESSING COMP-ANGIOPOIETIN-1 (F3.COMP-ANG1)

Chunggab Choi[1], Da-Jeong Chang[1], Seung-Hun Oh[1], Nayeon Lee[1], Hoosang Hwang[1], Hyun Sook Kim[1], Ok Joon Kim[1], Dong Ah Shin[2], Hyunseung Lee[3], Kwan Soo Hong[3], Seung U. Kim[4], Gou Young Koh[5], **Jihwan Song**[1]
[1]CHA Stem Cell Institute, Department Biomedical Science, CHA University, Seoul, Korea, Republic of, [2]Department of Neurosurgery, Yonsei University College of Medicine, Seoul, Korea, Republic of, [3]Division of Magnetic Resonance, Korea Basic Science Institute, Ochang, Korea, Republic of, [4]Medical Research Institute, Chung-Ang University College of Medicine, Seoul, Korea, Republic of, [5]Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of

Stroke is mainly caused by the obstacle of blood flow, leading to the ischemic condition which results in irreversible damages to the brain. Numerous efforts have been made, but there are still very limited therapeutic options for the stroke-damaged patients. In the stroke-damaged brain, cell death occurs not only in multiple neuronal and glial cell types, but also in endothelial cell lineages. Since the cross-talk between neural and vascular networks is very important for maintaining the homeostasis and proper function in the brain, we have paid attention to the roles of endothelial cells in the treatment of stroke. To do this, we have generated a human neural stem cell line overexpressing COMP-Angiopoietn-1 (F3.COMP-Ang1) using lentivirus. COMP-Ang1 is a soluble and stable form of Ang-1, which acts as a potent angiogenic factor. Cell characterization study indicated that F3.COMP-Ang1 cells express high levels of Ang1 and PECAM mRNA and protein, compared with F3 control cells. By contrast, expression levels

of Nestin remained largely unchanged. We also addressed the in vivo functions of F3.COMP-Ang1 cells in stroke-damaged rat brains by transplanting them into the contra-lateral side of cerebral infarct region and monitored the animals for 8 weeks. In vivo tracking study using a 4.7T animal MRI indicated that the transplanted cells were migrated to the infarct area extensively and gave rise to functional recovery. In particular, the transplanted animals exhibited significant behavioral improvements in the stepping, rotarod, staircase, apomorphine-induced rotation and modified neurological severity score (mNSS) tests. Histological analyses were carried out 8 weeks following transplantation. We initially found that the infarct volume of transplanted animals was significantly reduced, compared with sham controls, suggesting the neuroprotectve roles of F3.COMP-Ang1 cells. Immunohistochemical analyses further showed that the transplanted cells were positive for Nestin, NeuN, GFAP, PECAM and VEGF, indicating that they have the capacity to form both neural and endothelial lineages. They also expressed high levels of laminin, indicating the increased micro vessel density. We also found that the transplanted F3.COMP-Ang1 cells gave rise to significant reduction of inflammatory responses, gliosis and apoptotic cell deaths in the ischemic region, judged by the decreased expression of astrocyte marker (GFAP), microglia/macrophage-specific markers (Iba-1 and ED1) and TUNEL-positive cells. Taken together, these results strongly suggest that F3.COMP-Ang1 cells possess both neural and angiogenic properties, and they can also reduce inflammation, gliosis and apoptotic cell death. These unique properties of F3.COMP-Ang1 cells will provide a new therapeutic opportunity toward the treatment of stroke in the future.

This study was supported by a grant of the Korea Healthcare technology R&D project, Ministry for Health, Welfare & Family Affairs (A111016). We are grateful to the MRI facility in the Division of Magnetic Resonance, Korea Basic Science Institute, Ochang, Korea.

T-3256

ALTERED FATE DETERMINATION AND DIFFERENTIATION OF SUBGRANULAR ZONE NEURAL STEM CELLS AFTER STATUS EPILEPTICUS.

Matthew J. Korn, Jack M. Parent
Neurology, University of Michigan, Ann Arbor, MI, United States

Mesial temporal lobe epilepsy (mTLE) is a common and often intractable epilepsy which is known to produce long term changes within the subgranular zone (SGZ), the neural stem cell (NSC) niche in the adult hippocampus. It is hypothesized that newly generated neurons produced following epileptogenic insults contribute to hyperexcitability upon integrating into the established circuit. In the epileptic brain, adult-generated dentate granule cells (DGCs) exhibit ectopic migration, mossy fiber sprouting, and hilar basal dendrites, which are likely to contribute to aberrant plasticity. Additionally, disruption to the neural progenitor pool in chronic epilepsy may produce long term deficits in NSC self-renewal and neuronal maturation resulting in comorbidities such as depression, anxiety, and memory loss. To determine how SE affects neural stem cell fate determination, we evaluated the spatiotemporal distribution of type-1 and type-2 neural progenitors within the SGZ in an adult mouse chemoconvulsant-induced SE model of mTLE. Using antibodies to identify progenitor subsets we evaluated brains at 24 hours, 3, 7, 21, 28, 42 and 56 days after pilocarpine induced SE. Immunolabeling for Sox2, the Notch Intracellular Domain (NICD), glial fibrillary acidic protein and doublecortin (immature neurons) indicate that there is a dynamic shift in pro-neuronal gene expression that could underlie morphological and phenotypic alterations observed in mTLE. There was a notable increase in Sox2-expressing progenitors 24 hours after SE, followed by an overall decrease 21 days later. A similar phasic expression of NICD was observed in the SGZ, with levels returning to baseline 7 days post-SE. A decrease in Reelin signaling has been noted in human and experimental mTLE. Notch dependent expression of Hes5 is downstream of Reelin, therefore we also investigated whether the distribution of NICD is altered in Nestin-CreERT2/Rosa26YFP/Dab1(floxed) mice in which the Reelin effector disabled-1 (Dab1) is deleted in nestin-expressing progenitors. Results from these experiments suggest that NICD distribution plays an integral role at each phase of progenitor differentiation and that seizures may compromise the balance of the proliferating pool.

Ethics and Public Policy

F-1001

A MODEL POLICY FRAMEWORK FOR PRIVACY AND ACCESS TO INFORMATION CHALLENGES IN CELL THERAPY RESEARCH

Ubaka Ogbogu
Faculty of Law, University of Alberta, Edmonton, AB, Canada

Innovations in cell therapy research, such as induced pluripotent stem cells and triploid human embryonic stem cells, present vexing personal privacy and access to information challenges. These challenges include questions regarding the extent to which individuals involved in such studies can control the collection, use and disclosure of their personal genetic information for research purposes, and issues with keeping such information secure and confidential in a context where openness, data traceability, improved and easily accessible data re-identification technologies, and indeterminate future uses and applications of genetic research data, are fast becoming the norm. To date, no definitive responses to these challenges have emerged in academic and policy contexts. However, it is clear from existing scholarly and policy discourse that workable governance strategies are needed if the potential of and considerable financial investment in cell therapy research is to be realized without compromising privacy protections.
In March 2013, our research team will convene a multidisciplinary, multinational policy workshop in Banff, Alberta to deliberate on a model policy framework that addresses privacy and access issues associated with existing and emerging innovations in cell therapy and related research contexts. The framework is based on research undertaken with sponsorship from the Office of the Privacy Commissioner of Canada Contributions Program. This presentation will review the key issues and challenges revealed by our research, discuss the model policy framework, and reflect on the role and relevance of collaborative policy development activities in the context of dealing with governance challenges in cell therapy research.

F-1002

GLOBALIZATION OF STEM CELL SCIENCE: AN EXAMINATION OF CURRENT AND PAST COLLABORATIVE RESEARCH NETWORKS

Kirstin RW Matthews, Jingyuan Luo
Baker Institute for Public Policy, Rice University, Houston, TX, United States

In the past decade, science and engineering (S&E) research has become an increasingly international and collaborative endeavor. Recognizing that S&E research and development (R&D) leads to economic growth, increased employment, and overall social well-being, many national economies have intensified their investments and activities in the area. This has led to a shift in the geographic distribution of R&D expenditures, with Asia's share rising to 35% according to the 2012 National Science Foundation S&E Indicators.
Concurrent with this rise of global R&D activity is the increased number of internationally co-authored publications. Nearly one-fifth of medical and scientific papers now have international coauthors. Previous scholarship has demonstrated the benefits of collaborations, one of which - increased citation rates - that strongly incentivizes collaborative efforts. Historically, traditional factors including geographic proximity, language, and cultural practices have played a prominent role in a researcher's decision to collaborate. But as the worldwide research environment changes, it is increasingly important to examine the effects of emerging nations' presence in the international research environment. Traditional bibliometric studies have evaluated research trends by country, institute, journal, and field of study but have not captured the evolution of collaborative partnerships between countries.
Here we present a unique study that examines the extant research networks in one scientific discipline - stem cell science - and how they have changed in the past decade. Identified as one of the most important areas of biomedical research today, stem cell science is a field experiencing rapid growth in annual publications and patents. It is also an area of immense interests not only to scientists, but also to politicians and the public, as it is a heavily

publicized and often controversial topic. Analyzing nearly 2,500 articles from the top journals that include stem cell research in their publications, this study demonstrates the globalization of stem cell science. From 2000 to 2010, international collaborations increased from 22.3% to 36% of all stem cell publications analyzed. The United States remains the most prolific and the most dominant country in the field in terms of publications in high impact journals. But Asian countries, particularly China, are steadily gaining ground, although they still trail behind nations with a strong history of science and engineering research, such as those in North America. The data also indicated that traditional factors, such as geographic proximity and political ties, still correlate closely with the formation of international partnerships. After reviewing national stem cell policies, no immediate patterns correlating the degree of research permissibility with the formation of collaborative networks emerged, although policy may still be a factor in the decision to collaborate internationally. Overall, traditional networks of collaboration remain predominant in stem cell science, although more nations are becoming involved in international collaborations and undertaking stem cell research.

Society Issues

F-1004

MANAGING THERAPEUTIC HOPE AS A STRATEGY FOR COMBATING STEM CELL TOURISM

Insoo Hyun
Bioethics, Case Western Reserve Univ Sch of Medicine, Cleveland, OH, United States

Stem cell tourism involves the exploitation of desperate patients by unscrupulous commercial clinics advertising fraudulent claims about their proffered stem cell therapies. To date, the ISSCR and many commentators have attempted to address this problem through a range of strategies intended to reduce both the supply and the demand for stem cell tourism. To reduce the supply side, critics of stem cell tourism have advocated for greater local regulation of fraudulent stem cell clinics. To reduce the demand side of stem cell tourism, many have advocated increasing patient (and physician) awareness and education about the legitimate uses of stem cell biologics. In this presentation, I highlight the need to manage “therapeutic hope” as another important yet overlooked strategy in the fight against stem cell tourism.

Therapeutic hope is not the same as therapeutic misconception and therapeutic misestimation. The latter two involve errors in a patient’s understanding about the real or likely therapeutic benefits of a particular medical alternative. The cure for therapeutic misconception and misestimation is to increase the patient’s understanding to more realistic levels. In contrast, therapeutic hope involves not cognitive errors per se, but rather arises out of a patient’s emotional need to sustain his or her hopeful engagement with the future. Therapeutic hope is the psychological antidote to spiritual distress and fear that often accompany serious illness in the patient’s disease experience. The problem with therapeutic hope, however, is that, like therapeutic misconception and therapeutic misestimation, it can be easily exploited by unscrupulous stem cell clinicians to defraud patients.

While increasing patient understanding is the best prescription for therapeutic misconception and misestimation, providing more information alone is not likely to be the ideal prescription for managing therapeutic hope. Furthermore, therapeutic hope provides psychological benefits to the patient that should not be shrugged off or discouraged. This presentation offers realistic approaches to managing therapeutic hope while at the same time guarding against fostering “false hope” in patients. These realistic approaches draw on the professional recommendations of family physicians and medical social workers who have clinical experience dealing with therapeutic hope in patients, especially under circumstances of medical futility. This presentation urges medical professionals to combat the demand side of stem cell tourism by offering productive ways to channel patients’ therapeutic hope toward social support systems that can more helpfully sustain their hopeful engagement with the future. Managing therapeutic hope -- in combination with strategies aimed at increasing local regulations and improving patient understanding -- may provide a much more comprehensive approach to reducing the incidence of stem cell tourism in the future.

F-1005

MORE THAN OIL AND UPRISINGS: CURRENT DEVELOPMENTS IN UMBILICAL CORD BLOOD BANKING IN THE ARAB WORLD

Monica M. Matsumoto, **Kirstin RW Matthews**
Baker Institute for Public Policy, Rice University, Houston, TX, United States

Since the first successful therapy using umbilical cord blood (UCB) cells in 1988, UCB transplants have become an increasingly viable source for treating immune system and blood-related disorders and genetic diseases. UCB storage is a critical component of the treatment process enabling screening and preservation of the blood prior to its use in a matching patient. The United States created its first UCB bank in 1992, and numerous facilities, mostly in the United States and in Europe, have been established over the past 20 years. In the Arab countries of the Middle East, UCB banking remains in relatively early stages of development. As the potential extent of UCB therapy is still unknown, these nascent programs deserve attention from scientific, medical, ethical, and policy perspectives.
Recently, renewed investment has taken place in several Arab countries in the Middle East to improve scientific and medical infrastructure. Part of this priority has turned interest toward UCB transplantation and its potential in disease treatment. Currently, the region lags behind the United States and Europe in many aspects with regard to funding, regulations of procedures, quality standards, public awareness, and basic scientific research.
In this paper, we analyze the current state of UCB banking in several Arab countries of the Middle East. We will specifically highlight UCB banking in Jordan, Saudi Arabia, Qatar, Egypt, and the United Arab Emirates. These countries stand out in the region for their remarkable health care initiatives: Jordan dedicates the highest percentage of its GDP to health spending; Saudi Arabia, UAE, and Qatar have established ambitious programs for promoting medical, scientific, and technological innovation; and Egypt is the most populous country in the Middle East that, despite lacking many financial and scientific resources, has developed its own UCB program. All of these countries have recently turned their attention to the therapeutic uses of UCB and begun to invest heavily in banking and research programs, suggesting the potential of this new area of medicine. Our research will assess the extent to which UCB programs have been integrated in the medical infrastructure of these countries by taking into account the current state of research, accessibility of different types of UCB banks, and implementation of policies regulating UCB banking. We will also analyze the different approaches to UCB banking_public, private, and public-private hybrids_to determine which types are more prevalent in the region compared to the United States and Europe. Finally, we will address varying ethical and religious perspectives in the region associated with the donation, banking, and transplantation of UCB stem cells.

Cancer Cells

F-1011

RalA Signaling Pathway in Biology and Targeting of Cancer Stem Cells

Kaoru Terai[1], Kevin Ginn[2], Christopher Beaudoin[1], Kamyar Asadipooya[1], Zohreh Sanaat[1], Amanda L. Wise[1], Emma Borrego-Diaz[1], Daniel Ziazadeh[1], Aynur Agayeva[1], Tuba Esfandyari[1], **Faris Farassati**[1]
[1]Medicine, The University of Kansas-School of Medicine, Kansas City, KS, United States, [2]Pediatrics, Children Mercy Hospital, Kansas City, KS, United States

Although monoclonal in origin, most tumors appear to contain heterogeneous populations of cancer cells. One possible explanation of this tumor heterogeneity is that human tumors are not merely monoclonal expansions of a single transformed cell, but rather caricatures of normal tissues, and their growth is sustained by cancer stem cells (CSCs). Cancer Stem Cells are believed to be the main regenerative pool of cells in charge of repopulating tumors after exposure to therapeutic modalities. Therefore, gaining knowledge about the characteristics of CSCs, such as their signaling features, is important not only in terms of understanding the biology of tumors but also in developing novel anti-cancer therapies. Ras signaling pathway is an important pro-oncogenic signaling pathway involved in more than 40% of human cancers. We have evaluated the activation of Ras signaling pathway in a number of CSCs from different human tumor models such as cancers of lung, liver, ovary and peripheral nerve sheath in order to

understand if the activation of this pathway is different in CSC fraction. Markers of CSCs such as CD133, CD24 or CD44 were used to sort CSCs from a mixed population of cells. Different Ras down-stream pathways such as Erk, Jnk, p38 and RalA(Ras Like A) were evaluated in CSC fractions and compared with cells which were negative for or were expressing significantly lower levels of these markers. In general the activation of Ras down-stream effectors was higher in CSCs with RalA signaling being the constantly overactive effector among different tumor models. We have also used novel gene and drug therapy strategies in order to target RalA signaling pathway in these tumors resulting in significant loss of viability, invasiveness and in-vivo tumor growth. In some cases, a reversal of Epithelial-mesenchymal transition (EMT) was observed when RalA pathway was significantly inhibited. Therefore we introduce targeting RalA signaling as a major contributor to the biology of CSCs as well as a suitable target for attacking these cells.

F-1012

BCL11A: A NOVEL BREAST CANCER GENE WHICH PLAYS A CRITICAL ROLE IN MAMMARY STEM CELLS AND SUPPRESSES P53

Walid Khaled[1], Song Choon-Lee[1], Carlos Caldas[2], Pentao Liu[1]

[1]Wellcome Trust Sanger Institute, Cambridge, United Kingdom, [2]CRUK Cambridge Research Institute, Cambridge, United Kingdom

Basal-like breast cancer (BLBC) has the worst prognostic outcome among subtypes of breast cancer but driver mutations for this subtype are not well characterized. We have analysed genomics data from 2,800 patient samples (METABRIC and TCGA breast cancer datasets) and found the transcriptional regulator BCL11A to be overexpressed in 90% of BLBC cases and that its genomic locus is amplified in 20-40% of BLBC patients. In an experimental mouse model BCL11A overexpression in human breast epithelial cells caused BLBC-like tumours, whereas its knockdown blocked tumour development. To study the role of Bcl11a in normal mammary gland development we generated a LacZ knock-in mouse model. We found Bcl11a to be one of the earliest genes expressed during embryonic mammary development. In the adult mouse we found Bcl11a to be predominantly expressed in putative progenitor and stem cell compartments. To test its function in these cell compartments we generated conditional knockout (cKO) mice for Bcl11a. Fatpad transplant experiment revealed that deletion of Bcl11a in the mouse caused severe loss of mammary stem cell (MaSC) activities. At the molecular level, we show that BCL11A acts in part through its suppression of p53 activity through direct regulation of MDM2. Finally, we confirm genetically this novel interaction between Bcl11a and p53 by generating mice doubly deficient for both genes. Fatpad transplant experiments show that p53/Bcl11a double cKO cells can generate robust mammary outgrowth compared to no outgrowth from Bcl11a cKO cells, thus confirming the genetic interaction between p53 and Bcl11a in mammary stem cells. In conclusion, our data demonstrates the important role of BCL11A in BLBC pathology. Thus, BCL11A expression could predict accurate diagnosis and prognosis of BLBC patients in the clinic and its therapeutic targeting is warranted in the light of our findings.

F-1013

HUMAN PROSTATE CELL POPULATION DERIVED FROM BENIGN HYPERPLASIA SPECIMEN DEMONSTRATE PLURIPOTENT STEM CELLS PROPERTIES

Akhilesh Prajapati[1], Nidheesh Dadheech[1], Sharad Gupta[2], Sarita Gupta[1]

[1]Biochemistry, The M.S. University of Baroda, India, Vadodara, India, [2]Gupta Pathological laboratory, Vadodara, India

Abnormal prostate growth is most prevalent pathological sign in aged human males, reflected by high incidence of Benign Prostatic Hyperplasia (BPH) and Prostatic Carcinoma (PCa).The successful isolation and cultivation of prostate stem cells is a prerequisite need for establishing model cell line for understanding the pathogenesis; unique biological properties and their application in therapeutic approaches for benign and malignant prostate tumors.

Here we describe step-by-step procedures for the harvesting of primary prostate cells from BPH patients undergoing TURP which includes the isolation of an enriched population of prostate stem cells through cell sorting; and the cultivation of prostate stem cells in vitro and characterization of these cells and their stem potential, including in-vivo teratoma generation.Characterization of isolated cells showed presence of embryonic stem cell markers like Oct 3/4, Sox-2 and Nanog by mRNA expression, western blotting and flowcytometery. Further these cells were also found positive for CD49b, CD44, CD117, CD34 and prostatic tissue specific markers like p63 and Androgen Receptor. In-vitro differentiation of the cells demonstrated osteocyte, adipocyte, chondrocyte and neural cell lineage differentiation upon defined medium conditions and In-vivo teratoma formation in balb/c mouse with presence of tri-germinal layer representative in excised teratoma. Cytogenetic analysis by G-banding assay demonstrated an aneuploid karyotype with a model chromosome number of 60 (range 58 to 62, n = 20) with 4 to 5 marker chromosomes, which were structurally rearranged and the Y chromosome was found to be normal.
Conclusively, we report here isolation, establishment and characterization of human prostate-derived stem cell line. The cell line eventually serves as a potential tool for studies in prostate adult stem cell research, understanding etiopathophysiology and the regulation of Benign Prostatic Hyperplasia or prostate cancer.

F-1014

GATA3 ANTAGONIZES PROSTATE CANCER PROGRESSION

Mathieu Tremblay
Goodman Cancer Research Center, McGill University, Montreal, QC, Canada

Loss of the tumor suppressor PTEN is a common occurrence in prostate cancer. This aberration leads to the ectopic activation of the PI3K-Akt pathway, which promotes tumor growth. Here, we show that the transcription factor Gata3 is progressively lost in Pten-deficient mouse prostate tumors. Using both conditional loss- and gain-of-function approaches, we show that Gata3 inactivation in Pten-deficient prostates accelerates tumor invasion, whereas enforced expression of GATA3 in Pten-deficient tissues markedly delays tumor progression. Moreover, the dramatic increase in sphere-forming potential of Pten-deficient stem cells was reduced to wild-type levels by overexpression of Gata3 revealing a role for Gata3 in prostate stem cell homeostasis. This enforced expression of GATA3 prevented Akt activation associated with Pten loss, which correlated with the down-regulation of Pik3cg and Pik3c2a mRNAs, encoding respectively class I and II PI3K subunits. Remarkably, 75% of human prostate tumors similarly show loss of active GATA3 as they progress to the aggressive hormone-resistant stage. In addition, we identified high GATA3 expression levels in hormone-sensitive tumors (prior to castration) as a protection factor against cancer recurrence. Together, these data establish Gata3 as an important regulator of prostate cancer progression and point to a role for Gata3 in stem cell potential.

F-1015

FROM STEM/PROGENITOR CELLS TO TUMORS AND BACK TO CELL LINES: ESTABLISHING NOVEL MURINE PROSTATE CANCER CELL LINES FROM ANDROGEN-DEPENDENT AND -INDEPENDENT TUMORS.

Alissar Monzer[1], Rabih Merahbi[1], Paul Hynes[2], Yen-Nien Liu[3], Kathleen Kelly[2], **Wassim Abou-Kheir**[1]
[1]American University fo Beirut, Beirut, Lebanon, [2]National Institutes of Health, Bethesda, MD, United States, [3]Taipei Medical University, Taipei, Taiwan

Cell lines representing the progression of prostate cancer from an androgen-dependent to an androgen-independent state are scarce. Previously, we have established and characterized a new prostate luminal epithelial cell line (PLum), with *Pten/TP53* deletions, derived from a prostate epithelial stem/progenitor-enriched cell population. The deprivation of androgens from established PLum-orthotopic tumors resulted in tumor regression and eventually castration-resistant growth. Cells derived from orthotopic tumors have been isolated to develop androgen-dependent versus androgen-independent model. In this study, several experiments were conducted to establish and investigate the functional differences of the newly isolated androgen-dependent (PLum- AD) and androgen-independent (PLum-AI) prostate cancer cell lines. Unlike PLum-AD cells that grew in serum-free medium, PLum-AI cells

grew better in 2% FBS-containing medium. Both cell lines remained faithful in morphology to their in vivo source, where PLum-AD showed a typical epithelial morphology (in vivo source: adenocarcinoma) and PLum-AI showed an epithelial-to-mesenchymal morphology (in vivo source: sarcomatoid carcinoma). Furthermore, upon immunofluorescent analysis, PLum-AD cell expressed mostly prostate epithelial markers while PLum-AI cells expressed mesenchymal cell markers. In addition, QRT-PCR and Western blot analysis confirmed the epithelial and mesenchymal morphology of PLum-AD and PLum-AI respectively. To assess for the presence of stem/progenitor cell population, the cells were subjected to sphere-formation assay. Both cell lines had the capacity to form spheres, where PLum-AD cells formed regular-shaped spheres and PLum-AI cells formed mostly large stellate shaped spheres consistent with their mesenchymal-like nature. Interestingly, and despite no change at the mRNA level, AR protein expression level was higher in PLum-AD cells compared to PLum-AI cells. These data suggest that the newly isolated cell lines represent a new in vitro model of androgen-dependent and -independent prostate cancer where they recapitulate the progression of the disease to a more invasive phenotype upon androgen deprivation. More work has to be done to decipher the molecular mechanisms involved in this progression that would eventually lead us to new therapeutic targets.

F-1016

LYMPHOID PROGENITORS AND LEUKEMIC CELLS ISOLATED FROM PATIENTS WITH B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA ARE MAINTAINED BY MESENCHYMAL STEM CELLS

Viviana M. Rodríguez-Pardo[1], Jose A. Ariztizabal[1], Diana Jaimes[1], Sandra M. Quijano[1], Iliana de los Reyes[2], Maria V. Herrera[2], Javier Solano[2], Jean-Paul Vernot[3]
[A]Microbiology, [B]Hospital Universitario San Ignacio, [1]Universidad Javeriana, Bogota, Colombia, [2]Physiology, National University of Colombia, Bogota, Colombia

Hematopoietic stem cell (HSC) niches in the bone marrow (BM) regulate HSC and leukemic cell function by cellular and molecular mechanisms still poorly understood. Their study is increasingly relevant because their manipulation could be a promising therapeutic alternative. The role of human BM-mesenchymal stem cells (hBM-MSC) on leukemic cell control, is controversial. In this work, we have isolated hBM-MSC and have studied their effect on the maintenance, proliferation and immunophenotype of lymphoid progenitors (LP) and leukemic cells from B-cell acute lymphoblastic leukemia (B-ALL) patients. hBM-MSC were characterized by immunophenotype (CD34-/CD45-/ CD73+/ CD105+) and functional (ability to differentiate into osteo-, chondro- and adipocytes) criteria, and used as a feeder-layer for LP and B-ALL cells. We have shown that hBM-MSC can promote cell survival of both, normal and B-ALL cells, even after cell thawing. We have also found that BM-MSC can increase absolute cell counts and proliferation index (PI) of LP and B-LLA, after 7 days co-cultivation. Additionally, our results have shown that hBM-MSC increase CD19 expression on pre-pre-B cells and CD45 expression on Pre-B cells, but decrease CD19 expression on B cells. No changes in CD10 or CD20 antigen expression in LP cells were observed, after co-culturing. Likewise, hBM-MSC increase CD19 and CD45 expression in B-ALL, after 7 days of co-culture; however, in these cells, CD10 and CD20 expression was significantly increased after co-cultivation. We have demonstrated here that hBM-MSC promote maintenance of LP and B-LLA cells, but their effect on antigen expression was different, with B-LLA cells increasing CD19 and CD20 expression, while remaining stable in LP cells. The functional significance of these changes should be further studied. These findings can help to better understand the cellular interactions and their consequences in the leukemic niche.

F-1017

THE ROLE OF BONE MARROW DERIVED CELLS IN NODAL-ASSOCIATED NEOVASCULARIZATION

Dylan Dieters-Castator, Gilles Lajoie, Lynne-Marie Postovit
University of Western Ontario, London, ON, Canada

Acquisition of embryonic signalling pathways by tumor cells is associated with malignant phenotypes. Re-expression of the stem-cell factor Nodal in several cancers is one such example. Importantly, in breast cancer, Nodal is a

pivotal regulator of tumor vascularization, such that it is required for blood vessel recruitment and tumor growth in murine models and is positively correlated with high microvascular density in breast cancer patients. Moreover, bone marrow derived cells (BMDCs), including endothelial progenitor cells (EPCs) and mesenchymal stem cells (MSCs), incorporate into growing tumors and support neovascularization. To investigate the relationship between Nodal expression and BMDC recruitment and neovascularization in tumors, ALDHhi sorted human MSCs were initially examined for expression of Nodal signalling components. MSCs expressed transcripts and proteins for Nodal, its receptor ALK4, and co-receptor Cripto. Activation of the ALK4 receptor complex in response to treatment with recombinant human Nodal (rhNodal) with/without the small molecule ALK4/5/7 inhibitor SB431542 was determined by measuring downstream phosphorylation of Smad2 by Western blotting. Direct effects of Nodal on MSC chemotaxis and tube formation was investigated using transwell invasion assays and matrigel coated plates, respectively. Preliminary findings indicate that MSCs chemotaxis is not directly affected by rhNodal suggesting MSCs may respond indirectly to Nodal via proteins secreted by tumor cells. To address this possibility, MSC chemotaxis and tube formation was assayed in the presence of conditioned media (CM) from Nodal knockdown or overexpressing breast cancer lines (MDA-MB-231 and T47D backgrounds, respectively). CM from Nodal knockdown MDA-MB-231 cells was found to significantly reduce MSC invasion compared to controls. To characterize putative Nodal-regulated proteins involved in MSC chemotaxis, high-resolution, liquid chromatography-mass spectrometry was performed on breast cancer cell CM. Metabolic labelling of breast cancer cells by Stable Isotope Labelling with Amino Acids in Cell Culture (SILAC) enabled the quantification of relative changes in global protein expression. Several candidates known to be implicated in tumor progression were decreased in Nodal knockdown CM. Selection and validation of candidates and their effect on MSC phenotypes is currently being investigated. These studies aim to elucidate the mechanisms underlying Nodal-associated neovascularization in tumors involving BMDCs.

F-1018

A ROLE FOR THE TUMOR SUPPRESSOR PHF6 IN HEMATOPOIESIS

Finola E. Moore[1], Virginie Esain[2], Trista E. North[2], David M. Langenau[1]

[1]Molecular Pathology, Massachusetts General Hospital/Harvard Stem Cell Institute, Charlestown, MA, United States, [2]Pathology, Beth Israel Deaconess Medical Center, Boston, MA, United States

Plant Home domain Finger 6 (PHF6) is a tumor suppressor of unknown function in blood malignancies such as T-cell Acute Lymphoblastic Leukemia (T-ALL), Acute Myeloid leukemia (AML), and Chronic Myeloid Leukemia (CML). PHF6 contains two zinc finger-like PH domains and interacts with the Nucleosome Remodeling and Deacetylation (NuRD) complex, suggesting a role in chromatin remodeling. Although PHF6 mutations are found in nearly 40% of T-ALL patients, there is no loss of function animal model, little is known about how PHF6 mutations contribute to leukemogenesis. Hematological malignancies show marked overproliferation of immature blood cells in particular, suggesting that tumor cells have appropriated normal hematopoietic differentiation pathways. To understand the function of PHF6, beginning with its role in hematopoiesis, we have undertaken developmental studies in zebrafish to discover how loss of phf6 affects blood development and to determine which pathways are regulated by phf6. Zebrafish will be used to study hematopoiesis due to the remarkable conservation of molecular pathways that regulate blood development, genetic tractability, and ability to observe embryonic development over a short window of time. RNA in situ hybridization studies of zebrafish embryos showed that phf6 is expressed broadly during zebrafish development, and especially in the dorsal aorta, a site analogous to the aorta-gonad-mesonephros (AGM) in mammals, from which hematopoietic stem cells (HSCs) arise. Further, phf6 is highly expressed in myeloid and lymphoid cells of adult zebrafish, reminiscent of the expression patterns found in human and mouse. To determine the effect of phf6 loss on hematopoiesis, phf6 expression was knocked down by morpholino injection. We find that phf6 morphants have increased numbers of HSCs by RNA in situ hybridization of runx1/cmyb in the AGM and caudal hematopoietic tissue (CHT), sites of HSC emergence and migration. These results were confirmed by FACs of double positive HSCs from live transgenic Tg(runx1:GFP);Tg(lmo2:dsRed) animals. Similar phenotypes were observed in homozygous phf6-null mutants generated by TALEN-mediated knockout. In total, we found a new role

for phf6 in regulation of HSC formation, marking the first in vivo characterization of phf6 function. This work will provide powerful insights into the role of PHF6 in hematopoiesis and leukemia.

F-1021

CLONAL EVOLUTION DRIVES T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA RELAPSE THROUGH ACTIVATION OF THE AKT PATHWAY

Jessica Blackburn, Jayme Ranalli, Sarah Martinez, David Langenau
Massachusetts General Hospital, Charlestown, MA, United States

The aggressive and unpredictable behavior of relapsed T-cell acute lymphoblastic leukemia (T-ALL) presents a major clinical challenge, with >70% of children and >90% of adults unable to survive relapsed disease. In order to identify genes and pathways responsible for T-ALL relapse, we have developed a transgenic zebrafish model of relapsed T-ALL where single fluorescently-labeled cells are engrafted into genetically identical recipient fish and functionally assessed for differences in relapse growth. Using serial transplantation of single T-ALL cells and >6,000 adult transplant recipients, we have shown that 6 of 49 individual T-ALL clones undergo clonal evolution that increases their ability to form relapse. A subset of evolved clones that increased relapse potential also exhibited robust phosphorlyation of AKT at Ser473 resulting in
downstream pathway activation. Subsequent transgenic studies using a constitutively active myristoylated form of AKT confirmed that AKT pathway activation significantly increased the frequency of leukemia propagating cells by 8-fold and increased overall aggression/growth. Additional epistatic experiments and chemical genetic approaches revealed that AKT signaling plays three distinct roles in T-ALL relapse: 1) the AKT/mTORC1 pathway increases the frequency of tumor propagating cells capable of driving relapse, 2) AKT stabilization of Myc through phosphylation at Serine 62 enhances the proliferative rate of T-ALL cells, and 3) AKT pathway activation renders T-ALL cells unresponsive to dexamethasone, a cytotoxic chemotherapy commonly used to treat T-ALL. Building on these latter findings, we show that small molecule inhibition of AKT synergizes with dexamethasone in vivo to kill T-ALL cells that evolved drug resistance, suggesting that combination therapies that utilize PI3-kinase/AKT inhibitors and dexamethasone may provide new opportunities to treat relapsed and refractory disease. In total, our experiments identify AKT as a critical driver of relapse in T-ALL and are first studies performed in any model to follow single cell evolution as it relates to relapse, opening new and exciting avenues to uncover addition genetic and epigenetic pathways that drive cancer malignancy.

F-1022

THE PLURIPOTENCY FACTOR LIN28 IN PEDIATRIC AND ADULT LIVER CANCERS

Daisy A. Robinton[1], Marc Seligson[2], Liem Nguyen[3], Gen Shinoda[4], Samar Shah[5], George Q. Daley[2], Hao Zhu[6]
[1]Department of Biological Chemistry and Molecular Pharmacology, Harvard University, Boston, MA, United States, [2]Division of Pediatric Hematology/Oncology, Children's Hospital Boston, Boston, MA, United States, [3]Departments of Pediatrics and Internal Medicine, Children's Research Institute, University of Texas Southwestern Medical Center, Dallas, TX, United States, [4]Clinical Pharmacology/Asia/PC, Clinical Sciences, Bayer Yakuhin, Ltd, Osaka, Japan, [5]University of Pennsylvania, Pennsylvania, PA, United States, [6]Departments of Pediatrics and Internal Medicine, Children's Research Institute, University of Texas Southwestern Medical Institute, Dallas, TX, United States

Lin28 is a heterochronic RNA-binding protein that plays critical roles in developmental timing, pluripotency, tumorigenesis and metabolism. Though originally identified as a key regulator of development in *Caenorhabditis elegans*, its vertebrate homologs (Lin28a/b) have been found to be involved in a diverse array of physiological and pathological phenomena. Human LIN28A/B are highly expressed in subsets of many different types of cancer, but particularly prevalent in pediatric hepatoblastoma and adult hepatocellular carcinoma (HCC). LIN28B overexpression correlates with advanced disease, poor prognosis and lower survival rates in HCC. Lin28a/b modulate the biogenesis of the let-7 tumor suppressor miRNA family and affect translation efficiency of mRNAs. Recent evidence has

demonstrated a tight correlation between MYC overexpression, LIN28B activation (and less frequently LIN28A), and let-7 suppression in liver tumors. We are interrogating human HCC cell lines and two different MYC-driven models of HCC in mice to explore how Lin28a/b contribute to the progression of liver cancer. In mice, Lin28b is expressed at high levels in the developing liver (E14.5), then drops to low levels in early postnatal life. Conditional induction of MYC during embryonic development results in neonatal liver tumors reminiscent of pediatric hepatoblastoma, and like the human disease, we have documented overexpression of Lin28b accompanied by a significant down-regulation of let-7 miRNAs in murine tumors. Direct mRNA binding targets of Lin28, such as GLUT4 and IGF2, as well as let-7 targets such as IMP1, are up-regulated in these neonatal liver tumors. Later induction of MYC in adult life results in HCC, but relative to neonatal tumors, the tumors that form in adulthood express lower levels of LIN28 protein. Additionally, we have demonstrated that knockdown of LIN28B compromises survival and proliferation of human HCC cell lines *in vitro*. Our data implicate Lin28 activation in liver tumorigenesis. Using engineered strains of mice that allow conditional overexpression or deletion of Lin28a and/or Lin28b, we are currently executing gain and loss-of-function studies to test the necessity and sufficiency of Lin28a/b expression for tumor initiation and maintenance.

F-1023

DIFFERENTIAL RESPONSES OF LUNG STEM CELLS AND TUMORS TO EZH2 DELETION

Christine M. Fillmore, Carla Kim
Stem Cell Program, Boston Children's Hospital, Boston, MA, United States

EZH2 is a histone methyltransferase which plays roles in embryonic stem cells, adult stem cells and cancer. In cancer, as well as in normal tissue stem cells, EZH2 may act to promote growth and self-renewal, or to promote cell cycle arrest and differentiation in different cellular contexts. However, a model that directly tests long and short-term effects of EZH2 depletion on stem cell and tumor cell activities has yet to be established. To explore the roles of EZH2 in lung stem cell function and during lung tumor initiation, we used the EZH2 conditional (floxed) mice. We first used a doxycycline inducible Cre system to deplete EZH2 in many cell types within the lung. When assayed 4 days after doxycycline administration, EZH2fl/fl bronchioalveolar stem cells (BASCs) were 2.8-fold less competent to form in vitro colonies when compared to EZH2+/+ BASCs. Similarly, EZH2fl/fl basal tracheal stem cells showed 2.4-fold less tracheosphere formation, and 6.5-fold less tracheosphere self-renewal. Short term EZH2 deletion also significantly attenuated CCSP+ cell regeneration in the distal lung after napthalene injury. In contrast, EZH2fl/fl BASCs and basal cells isolated from mice that received 4 months of doxycycline showed significantly more colony formation than those from 4 day mice, suggesting a selection of stem cell populations with increased fitness in response to longer term EZH2 depletion. We next tested the effects of EZH2 deletion on lung adenocarcinoma initiation using the Kras/p53 mouse model. Kras/p53/EZH2fl/+ animals given intranasal Adeno-Cre had 29% tumor burden, compared to the 63% tumor burden seen in Kras/p53/EZH2+/+ mice. Unexpectedly, Kras/p53/EZH2fl/fl mice had increased tumor burden and metastasis when compared to Kras/p53/EZH2+/+ mice. These data suggest that both the level and duration of EZH2 depletion can significantly affect the response to stem cells and tumor cells to loss of this protein.
This work was supported by LAVFW and ACS grant 123438-PF-12 to C.M.F.

F-1024

A MOUSE MODEL OF BREAST CANCER SPONTANEOUS METASTASIS USING HUMAN PRIMARY CANCER CELL LINE WITH HIGHLY CANCER STEM CELL CHARACTERS

Ruey-Jen Lin, Wen-Ying Chang, Alice L. Yu
Genomic Research Center, Academia Sinica, Taipei, Taiwan

Metastasis is the main cause of death in women with breast cancer. However the study of breast cancer metastasis has been limited by lacking reliable metastatic models. The purpose of this study was to develop a novel and reproducible breast cancer model using established primary cell lines isolated from patient tumor mass for the in-

vestigation of the metastatic process. By using primary cells with highly cancer stem cell (CSC) properties of tumor formation capacity and aldehyde dehydrogenase (ALDH) activity to xenograft orthotopically into the mammary fat pads of NON/SCID and NOD scid gamma (NSG) mice, Macro-metastases were frequently and consistently observed in iliac lymph nodes, axillary lymph nodes and lungs. In this model human breast cancer cells metastasize to distant organs from primary tumors grown within the mammary fat pad were more efficiency than well known breast cancer cell lines xenografts, hence enable to study of metastatic process as well as to exam the role of CSC in breast cancer metastasis. This mouse model will provide more powerful system to elucidate the underlying mechanisms of metastasis and tumor progression, and to introduce drugs targeted to individual cancer patients.

F-1025

TARGETING THE COX/β-CATENIN SIGNALING PATHWAY IMPAIRS AML SELF-RENEWAL AND LEUKEMOGENESIS

Joanna Yeh, Yiyun Zhang, Jianfeng Wang, Justin Wheat, Xi Chen, Shan Jin, Randall Peterson, David Sweetser
Massachusetts General Hospital, Charlestown, MA, United States

Investigating oncogenic effects in the multipotent hematopoietic stem/progenitor cells (HSPCs) of zebrafish embryos may reveal the key signaling pathways governing leukemia stem cells (LSCs). We have developed a zebrafish model expressing the human leukemia oncogene AML1-ETO. Expression of AML1-ETO in embryonic zebrafish immediately induces hematopoietic differentiation defects mimicking human acute myeloid leukemia (AML). Via a compound library screen for chemical suppressors of the AML1-ETO phenotypes in zebrafish, we have uncovered the surprising roles of a COX-2- and β-catenin-dependent pathway in AML1-ETO function. We show that AML1-ETO upregulates the Cox2 gene, which activates β-catenin signaling in mouse HSPCs. Inhibition of COX suppresses β-catenin activation and serial replating of AML1-ETO+ mouse HSPCs. This is due to a significant reduction of the AML1-ETO+ hematopoietic stem cells (HSCs), not an overall increase of apoptotic cells. Genetic knockdown of β-catenin also abrogates the clonogenic growth of AML1-ETO+ mouse HSPCs and human AML cells. In addition, treatment with a COX-2 selective inhibitor dramatically suppresses xenograft tumor formation and inhibits in vivo progression of human AML cells in mouse models without affecting mouse HSCs. In summary, our data indicate the important roles of a COX/β-catenin pathway in AML initiation, growth and self-renewal. Interestingly, data from other groups suggest that this pathway also plays a critical role in AML associated with other oncogenes and in chronic myeloid leukemia. Thus, incorporating common COX inhibitors as a part of AML treatment regimen may suppress self-renewal of LSCs, reduce the likelihood of relapses and extend long-term survival of patients with AML.

F-1026

THE ROLE OF CANCER STEM CELLS IN OSTEOSARCOMA

Andrew D. Jenks, Mark Clements
School of life sciences, University of Westminster, London, United Kingdom

Osteosarcoma (OS) is the most common bone malignancy often producing aggressive tumours which mainly effect adolescents. OS aetiology is poorly understood, however, recent studies suggest OS cancers contain a small population of cancer stem cells (CSC) which initiate tumour growth. The CSC hypothesis describes cancers as a hierarchical population of heterogeneous cells. It has been proposed that CSC are at the base of this hierarchy and are responsible for the initiation, growth and spread of the tumour and pose a therapeutic challenge due to enhanced chemotherapy resistance.
This study has three objectives: to characterise markers of OS CSC, determine the role of OS CSC in chemotherapeutic resistance and to elucidate paracrine cell signals controlling OS tumour growth. The study used eight established OS cell lines ensuring the results are representative of OS in general.
The results confirm that OS cell lines contain a sub-population of cells expressing the OS CSC markers aldehyde dehydrogenase and CD117. All cell lines were able to replicate a morphological CSC colony hierarchy (holoclones, meroclones and paraclones) which is further evidence of CSC in OS. Varying degrees of chemotherapy (methotrexate) sensitivity were observed between cell lines. The high expression of CSC markers did not correlate with altered

chemotherapy resistance. The OS cell line U2OS was found to secrete a paracrine factor that enhanced both the growth and presence of CSC in OS cell lines and also the breast cancer cell line MCF7.
In conclusion OS contains a subpopulation of cells with features of CSC. The CSC do not display increased chemotherapeutic resistance to methotrexate. OS cells produce a paracrine growth factor which increases not only the growth but frequency of CSC in both sarcomas and breast cancer and may form a novel therapeutic target.

F-1027

COMPUTATIONAL CORRELATION AMONG MICRORNAS AND MRNAS SPECIFICALLY EXPRESSED IN PANCREATIC CANCER STEM CELLS IN VITRO AND ITS POSSIBLE APPLICATION IN PANCREATIC CANCER DIAGNOSIS

Dawoon Esther Jung[1], Kyong Joo Lee[1], Hyungseok Choi[2], Seungmin Bang[1], Seung Woo Park[1], Si Young Song[1]
[1]Division of Gastroenterology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea, Republic of, [2]LG Electronics, LGE Advanced Research Institute, Seoul, Korea, Republic of

Objective: The objective of this study was to analyze and identify pancreatic cancer stem cell-specific microRNAs (miRNAs) and messenger RNAs (mRNAs) and investigate their correlations to cancer stem cell biology. More, expression of pancreatic cancer stem cell-specific miRNAs in cancer patients' serum was analyzed to determine as a diagnostic application.
Methods: We used sphere cultivation methods to enrich the stem cell population from the pancreatic cancer cell line, Capan-1 with the normal pancreatic cell line, YGIC-6, and analyzed the overall miRNAs and mRNAs expression using with microarray analysis. Total RNA from normal and patient's serum was extracted and the expression individual miRNAs in serum was analyzed by real-time PCR.
Results: Fifty two miRNAs including mir-26a, miR-99a, miR-106a, mir-125b, miR-192 and mir-429 were differentially expressed in the pancreatic cancer stem cells. From the miRNA analysis, 170 miRNAs were differentially expressed in the normal cell line YGIC-6, 126 miRNAs in the cancer cell line Capan-1 and 12 miRNAs in both the normal and cancer cell line originated stem cell populations. Examining both the miRNA and mRNA profiles, 52 miRNAs and 111 stem cell-associated mRNAs were highly correlated (both p values<0.01) that were differentially expressed in the pancreatic cancer stem cells. These miRNAs and mRNAs were further investigated with cross-correlation analysis, which showed a number of highly correlated miRNAs and mRNAs that are either directly or indirectly linked based on the target prediction software. The correlation degree is shown on the heatmap, and individual miRNAs with their highly correlated individual mRNAs were analyzed to predict possible mechanisms for miRNAs in pancreatic cancer stem cells. To examine miRNAs level in serum, total RNA was extracted from normal(n=8) and cancer patient's serum(n=20) and the expression level of individual miRNAs were analyzed. The expression level of mir-106a and mir-125b was 10-fold, and 2- fold lower and mir-429 was 2.3-fold higher in cancer patient's serum than normal.
Conclusions: Differentially expressed miRNAs in pancreatic cancer stem cells provide insights into possible linkages between individual miRNAs and stem cell-associated target mRNAs in cancer stem cells and have broad implications in our understanding of cancer stem cells and cancer stem cell-targeted cancer therapy. Expression level of miRNAs in serum differentiated normal and cancer patient which suggests miRNAs as a potential diagnostic marker.

F-1028

REPROGRAMMING OF THREE PANCREATIC EXOCRINE CANCER CELL LINES AND THEIR CHARACTERIZATION

Ayana Sakurai[1], Yoshie Susumu[1], Tomotsune Daihachirou[1], Gaku Houmei[1], Sasaki Katsunori[1], Yokoyama Tadayuki[2], Takizawa Sakiko[2]
[1]Histology and embryogy, Shinshu University School, Matsumoto, Japan, [2]BOURUBON Corporation, Matsumoto, Japan

It is well-known that it is very difficult to diagnose and remedy pancreatic exocrine cancers, and most affected individuals are fatal. To defeat these diseases, we map out the following strategies: to know how they proceed and

what metabolism occurs in them and to find what kind of markers they produce during its progression. As a tactics, we choose to generate iPS cells from pancreatic exocrine cell lines, because they do not lose their carcinomatous memories completely and are expected to progress into cancer from the first stage again. The four transcription factors, C-myc, Oct3/4, KLF4, and Sox2, were transfected into three pancreatic exocrine cancer cell lines, Panc-1, Bx-PC3, and Mia-pa-caII, with ecotropic retrovirus produced in PLAT-E packaging cells. Infected Panc-1 and Bx-PC3 formed several colonies on day 14 to 18, but Mia-pa-caII did not. Eight colonies of Panc-1 and Bx-PC3 were picked up, proliferated, and analyzed using cell biological methods. Several colonies from Panc-1 indicated the expression of Nanog.
However, the expression of Sox2 and Rex-1 were weakly detected in there colonies. On the other hand, colonies from Bx-PC3 expressed definitely Sox2, but weakly Nanog and Rex-1. Interestingly, the expression of PDX-1 was higher in all colonies than control cell line, which were not transfected. From these results, our findings raise the possibility that pancreatic cancer cell dedifferentiated cancer stem cell. In this paper, adding DNA microarray analysis to these observations, gene expression features of pancreatic cancer cell-derived cells colonies from cancer cell lines will be discussed.

F-1031

TARGETED THERAPY OF LEUKEMIA STEM CELLS IN PTEN-DEFICIENT T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL)

Anjelica M. Cardenas[1], Suzanne Schubbert[2], Consuelo D. Garcia[2], Harrison Chen[2], Cindy Malone[1], Hong Wu[2]
[1]Biology, CSU-Northridge, Northridge, CA, United States, [2]Pharmacology, UCLA, Los Angeles, CA, United States

T-cell acute lymphoblastic leukemia (T-ALL) is a hematological malignancy associated with significant risk of relapse. Deletion and mutation of the tumor suppressor gene, phosphatase and tensin homolog *(PTEN),* has been reported in a large percentage of pediatric patients with T-ALL and associated with poor prognosis. Our laboratory has developed a *VEC-Cre*$^{+}$;*Pten*$^{loxp/loxp}$ (*Pten* null) T-ALL mouse model to investigate the mechanisms underlying T-ALL pathogenesis and resistance to therapy. Our previous studies have shown the PI3K/AKT/mTOR pathway inhibitor, rapamycin, suppresses T-ALL development in pre-leukemic *Pten* null mice. However, rapamycin cannot eradicate leukemia stem cells (LSCs) and abolish T-ALL in mice with acute disease. Since rapamycin is not sufficient to eliminate LSC, we hypothesize that utilization of combination therapy to target critical molecular alterations and deregulated pathways required for LSC formation and T-ALL development may be a more effective approach to eliminate LSCs and T-ALL. Because Myc over-expression is required for LSC formation and leukemia development in *Pten* null T-ALL, we plan to target c-Myc over-expressing cells with Myc inhibitors, VX-680 and JQ1. VX-680 is an Aurora kinase inhibitor that blocks cell cycle progression reported to induce apoptosis and autophagy in Myc over-expressing cells. JQ1 is a protein bromodomain-containing 4 (Brd4) inhibitor shown to down regulate Myc expression in cells. In this study we utilize our recently derived *Pten* null T-ALL *in vitro* cell culture to evaluate the therapeutic utility and biochemical mechanisms underlying the effects of synthetic Myc inhibitors as single agents and in combination with rapamycin. Cell proliferation assays were performed and we found cell viability significantly decreased in *Pten* null cells treated with VX-680 and JQ1. In contrast to our *Pten* null cell line, Jurkat cells of an established human T-lymphocyte line show little sensitivity to rapamycin, VX-680, and JQ1 treatment, even at high doses. Cell cycle analysis showed VX-680 treatment caused a robust increase in sub G_0/G_1 apoptotic population following treatment in *Pten* null cells. We found that VX-680 does not affect DNA synthesis and caused formation of a polyploid (8N) population followed by induction of apoptosis. Biochemical analysis shows VX-680 treatment also induces levels of phospho-histone γH2A, indicating a DNA damage response, and results in increased levels of cleaved caspase 3 and cleaved PARP, consistent with apoptosis. JQ1 caused robust down-regulation of the IL-7 receptor, a down-stream target regulated by Brd4, suggesting JQ1 is hitting target in *Pten* null cells. Because our *Pten* null cells were sensitive to VX-680 and JQ1 treatment *in vitro*, we are investigating the ability of VX-680 and JQ1 to eliminate LSC population and T-ALL in our *Pten* null T-ALL mouse model. Our preliminary data suggest that combination treatment using rapamycin and VX-680 or rapamycin and JQ1 causes robust elimination of blasts and more importantly, reduced LSC populations *in vivo*. Our results suggest that co-targeting the PI3K/AKT/mTOR pathway and Myc over-expres-

sion eliminates LSCs and T-ALL more vigorously than rapamycin alone. These studies highlight the importance of understanding the molecular mechanisms underlying T-ALL development, show potential of targeted combination therapies to kill LSCs and abolish T-ALL, and offer a possible, improved therapeutic strategy to treat human T-ALL.

F-1032

TOX: A NOVEL ONCOGENE IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

Riadh Lobbardi[1], Nouran Abdelkader[2], Manon De Waard[3], Jessica S. Blackburn[1], David M. Langenau[1]

[1]Molecular Pathology/Cancer Center Research, Massachusetts General Hospital/Harvard Medical School, Charlestown, MA, United States, [2]Worcester Polytechnic Institute, Worcester, MA, United States, [3]University of Leiden, Leiden, Netherlands

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy of thymocytes with cMyc being a potent oncogene in vast majority of T-ALLs. High mortality rates associated with chemotherapeutic drug resistance have led to a great need for more effective compounds for the treatment of this disease. It is therefore important to identify novel drug targets and new molecular pathways underlying T-ALL progression. To this end, we have recently completed a large-scale zebrafish transgenic screen to identify functionally relevant genes that collaborate with cMyc to shorten time to tumor onset. Specifically, 38 amplified and over-expressed genes found in human relapsed T-ALL were assessed for collaboration in the zebrafish T-ALL model. From this analysis, we identified Thymocyte Selection-associated high mobility group box (TOX) as a potent collaborator with the Myc oncogene. TOX is important for the specification of CD4 cells, maturation of the CD8 cells, and myeloid cell development. However, a role for Tox in regulating T-ALL is unknown. Importantly, we find that TOX is over-expressed in at least 15 human T-ALL cell lines, is highly expressed in primary tumors, and has been reported to be amplified in both human and mouse T-ALL. To determine whether TOX plays a crucial role in T-ALL maintenance and growth, shRNA knock down studies were completed in human HPB-ALL, CCRF-CEM, and MOLT-4 cell lines. shRNA knockdown resulted in decreased cell viability reflected by elevated apoptosis and cell cycle arrest in the late S- phase. Because TOX is likely a non-specific DNA binding factor that lacks the ability to drive transcription, we hypothesized that TOX might be a scaffolding protein that tethers proteins to DNA. Endogenous TOX was pulled down using a specific antibody and Tandem Mass Spectrometry was completed to identify interacting factors. We found that Ku70 and Ku80 are immunoprecipated with TOX. This interaction was confirmed by reciprocal pull downs using antibodies to Ku70 and Ku80 and was completed in the presence of DNAse, confirming that protein associations were specific and not mediated merely by common binding to DNA. Ku70 and Ku80 are recruited to double strand breaks as the first step in non-homologous end joining (NHEJ) and have important roles in TCR-α rearrangement. Intriguingly, Ku70-deficient mice are predisposed to T-cell lymphoma and exhibit a profound cell cycle arrest in the late S-phase, suggesting that TOX may be a negative regulator of Ku70/ku80 function. We are now investigating the role of TOX in regulating DNA repair processes. The ultimate goal of this study is to understand how TOX inhibits the DNA damage repair pathway with the hope of identifying drugable pathways to suppress TOX function.

F-1033

PLEIOTROPIC EFFECTS OF TUMOR SECRETED FACTORS ON HUMAN MESENCHYMAL STEM CELL

Abdulaziz A. AlMusa[1], Mashael Al-toub[1], Mohammed Almajed[1], May Al-Nbaheen[1], Moustapha Kassem[2], Abdullah Aldahmash[1], Nehad M. Alajez[1]

[1]Stem Cell Unit, Department of Anatomy, King Saud University, Riyadh, Saudi Arabia, [2]Department of Endocrinology, University of Southern Denmark, Odense, Denmark

Objective: Recent years has witnessed huge interest in studying the tumor microenvironment, given its apparent role in driving tumor progression and metastasis. Of particular interest, mesenchymal stem cells (MSCs) have been the focus of many research groups as the exact role of MSCs in driving cancer progression remains con-

troversial. Herein, we investigated the effects of tumor secreted factors from a panel of human cancer cell lines (breast (MCF7 and MDA-MB-231); prostate (PC-3); lung (NCI-H522); and head & neck (FaDu)) on MSCs.
Methods: Morphological changes were assesses using fluorescent microscopy. Changes in gene expression were assessed using Agilent microarray and qRT-PCR. Cell migration was assessed using transwell migration system.
Results: Morphologically, MSCs exposed to secreted factor from FaDu, MDA-MB-231, PC-3, and NCI-H522, but not from MCF7, exhibited a spindle-shaped morphology, and the cells were more elongated with bipolar processes, compared to control MSCs which were larger and more flattened with multiple processes. Integrated analysis of gene expression and bioinformatics revealed a proinflammatory response of MSCs when exposed to conditioned media (CM) from all cell lines, but not MCF7. Nonetheless, MSCs exhibited significant tropism toward secreted factors from the aforementioned tumor cell lines.
Conclusions: Thus our data suggest that MSCs might drive tumorigenicity through induction of inflammation.

F-1034

ISOLATION AND PHENOTYPIC CHARACTERIZATION OF EPITHELIAL OVARIAN CANCER STEM-LIKE CELLS

EUN JIN SEO
Pusan National University, Yangsan-si, Korea, Republic of

Multiple studies in the recent years have identified highly tumorigenic populations of cells that drive tumor formation. Cancer stem cells (CSCs) are reportedly implicated in drug resistance, recurrence, and metastasis of various tumors. Markers that specifically recognize CSCs can be applied to the diagnosis and treatment of ovarian cancer. Several markers have been used for the identification of epitherial ovarian cancer(EOC) stem cells, which reflects the heterogeneity of ovarian cancer. These markers include CD44, CD24, CD133, MyD88 and CD117. Among these markers, aldehyde dehydrogenase1(ALDH1) can be a promising marker of CSC. In this present study, we isolated spheroid forming CSC-like cells from A2780, a human epithelial ovarian cancer cell line. The spheroid-derived cells displayed the self-renewal potential and the elevated expression of ALDH1. They also showed higher invasiveness, more migration, and the enhanced resistance to standard anticancer agents(paclitaxel,cisplatin) than their parental cancer cells. These results suggest that ALDH1 is a good marker for isolating ovarian cancer spheroid-forming cells, which can be a useful platform for the study of ovarian CSCs and the development of novel CSC-targeted therapies.

F-1035

PHENOTYPIC PLASTICITY OF TUMOR- INITIATING CELLS IN HUMAN COLON CANCER

Taronish Dubash[1], Christopher Hoffmann[1], Claudia Ball[1], Felix Oppel[1], Sebastian Dieter[1], Wilko Weichert[2], Jürgen Weitz[3], Martin Schneider[3], Moritz Koch[3], Manfred Schmidt[1], Christof von Kalle[1], Hanno Glimm[1]
[1]Translational Oncology, National Center For Tumor Diseases (NCT) and German Cancer Research Center (DKFZ), Heidelberg, Germany, [2]Institute of Pathology, University of Heidelberg, Heidelberg, Germany, [3]Department of Surgery, University of Heidelberg, Heidelberg, Germany

We have recently shown that a small subfraction of all tumor initiating cells (TIC), extensively self-renewing long-term TIC, drive long-term progression and metastasis formation of human colorectal cancer in immunodeficient mice. It remained unknown whether LT-TIC activity is associated with a fixed phenotype allowing their prospective isolation.
To address this question we enriched TIC from primary human colon cancer samples in serum-free spheroid cultures in the presence of FGF and EGF. Decreasing numbers of spheroid cells were transplanted into cohorts of Nod/SCID-IL2RG$^{-/-}$ (NSG) mice to calculate the frequencies of TIC. Frequencies varied between cultures from different patient samples between 1 in 22 spheroid cells and 1 in 2x10^4. To induce a more differentiated phenotype 10% fetal calf serum was added, cytokines were withdrawn and cells were transferred into gelatin coated dishes to support cell adherence. Under these conditions, the cells formed a morphologically differentiated-like adherent cell layer. The morphological changes were fully reversible when the cells were transferred back into serum-free spheroid

culture conditions. Differential gene expression analyses demonstrated upregulation of the colonic differentiation markers CDX2, DEFA5, KRT80, Muc20 and TFF2 in phenotypically differentiated cultures as compared to serum-free spheroid cultures. In contrast, the cell surface protein CD133, a marker commonly used to enrich for TIC in different solid tumors was strongly down regulated. Strikingly, after transplantation the primary human colon cancer cells from spheroid cultures and cultures favoring differentiation equally well formed tumors in NSG mice with similar histology. Secondary transplantation experiments demonstrated that the self-renewal capacity of TIC was unaltered by differentiation promoting culture conditions. CD133 expression did not predict tumorigenicity in vivo. Sorted CD133+ and CD133- cells from 3 individual patient cultures formed tumors with equal efficiency and regenerated both CD133+ and CD133- cells in vivo.
In summary, our results demonstrate that phenotypic differentiation in vitro does not affect the tumor-initiating potential of human colorectal TIC and that the expression of CD133 on TIC reversibly varies without close association to their tumor forming or self-renewal potential. This pronounced phenotypic plasticity of human colon cancer TIC poses a serious challenge for the development of strategies specifically targeting this highly relevant cell population in colorectal cancer.

F-1036

INFLUENCE OF SYSTEMIC TRANSPLANTATION OF ALLOGENEIC OR XENOGENEIC BONE MARROW-DERIVED MESENCHYMAL STEM CELLS ON THE GROWTH OF TRANSPLANTED RAT TUMOR M-1 AND ITS REACTION ONTO THE RADIATION THERAPY

Vadim Yuzhakov[1], Larisa Sevankaeva[1], Mikhail Konoplyannikov[2], Anatoly Konoplyannikov[1]
[1]Medical Radiological Research Center, Obninsk, Russian Federation, [2]Federal Research Clinical Center, Moscow, Russian Federation

In this study, we have studied the morpho-functional effects of systemic (intravenous) transplantation of allogeneic or xenogeneic mesenchymal stem cells (MSC) to white wildtype rats with the previously transplanted M-1 sarcoma. It has been found that the intravenous infusion of bone marrow-derived human or Wistar rats MSC (10^6 cells per 100 g of the rat weight) has a marked effect on the growth of the transplanted tumor. After a short initial stimulation of the tumor growth in the early times after the MSC infusion, a tendency to the subsequent tumor growth deceleration was detected. A response of the transplanted tumor to the local γ-irradiation in the dosage of 30 Gr was also changed after the MSC transplantation. We estimated this response by the times of the animals' death and also by the changes in the neoplasms morphology on the 3^d and 12^{th} days after the irradiation. The methods of study included immunohistology testing on PCNA, measurements of mitotic activity and apoptotic death of tumor cells, and also morphometry of the regions of spontaneous and induced necrosis and remaining parenchyma of the M-1 sarcoma. It was found that MSC transplantation 4 days prior to the local tumor irradiation extended the life expectancy of animals and enhanced the morphological indices of the radiation damage of the tumor. We concluded that transplantation of both allogeneic and xenogeneic MSC resulted in the improvement of the peritumoral angiogenesis and increase of the total fraction of proliferating tumor cells; the radiosensitizing effect of MSC administered before the irradiation was generally related to the reduction in the fraction of most resistant hypoxic cells in the region of the radiation action onto the tumor. These data, after being confirmed in the experiments on other tumor types, may become a basis for development of new methods of radiosensitization of resistant malignant neoplasms.

F-1037

C/EBPα INDUCES HIGHLY EFFICIENT MACROPHAGE TRANSDIFFERENTIATION OF SELECTED B-LYMPHOMA AND LEUKEMIA CELL LINES AND IMPAIRS THEIR TUMORIGENICITY

Francesca Rapino[1], Eloy F. Robles[2], Jose A. Richter-Larrea[2], Eric M. Kallin[1], Jose A. Martinez-Climent[2], Thomas Graf[1]
[1]Centre for Genomic Regulation (CRG), Barcelona, Spain, [2]Division of Oncology, Center for Applied Medical Research, University of Navarra,Pamplona, Spain, Spain

Earlier work demonstrated that the transcription factor C/EBPα can convert immature and mature murine B lineage cells into functional macrophages. Testing >20 human lymphoma and leukemia B-cell lines we found that most can be transdifferentiated at least partially into macrophage-like cells, provided that C/EBPα is expressed at sufficiently high levels. A high level C/EBPα subclone of a Burkitt lymphoma line, corresponding to mature B-cells, could be efficiently converted into phagocytic and quiescent cells with a transcriptome resembling normal macrophages. The converted cells retained their phenotype even when C/EBPα was inactivated, a hallmark of cell reprogramming. Interestingly, C/EBPα induction in vitro or in vivo also impaired the cells' tumorigenicity. Likewise, C/EBPα efficiently converted a B- lymphoblastic leukemia cell line into macrophage-like cells, and these were dramatically impaired in their tumorigenicity. Our experiments show that selected human cancer cells can be induced to transdifferentiate into seemingly normal cells at high frequency and provide a proof of principle for a potential new therapeutic strategy to treat B-cell malignancies.

F-1038

TARGETING BOTH ACTIVE AND QUIESCENT CANCER (STEM) CELLS USING COMBINED THERAPY

Xi He[1], Xiazhen Yu[2], Ryohichi Sugimura[1], Fengchao Wang[1], Meng Zhao[1], Fang Tao[1], Donghua Liu[1], Brandy Lewis[1], Andrew Box[1], Mark Hembree[1], John Perry[1], Ed Lin[3], Linheng Li[1]

[1]Stowers Institute for Medical Research, Kansas City, MO, United States, [2]Second Affiliated Hospital School of Medicine, Zhejiang University, Hangzhou, China, [3]Division of Medical Oncology, Seattle Cancer Care Alliance, Seattle, WA, United States

The conventional approach of targeting proliferating cells has largely been unsuccessful in curing cancers and this could most likely be attributed to not targeting cancer stem cells (CSCs), the 'seed' of malignancy. We and others have hypothesized that akin to normal stem cells, cancer stem cells (CSCs) may have adopted dual active-quiescent mechanisms to sustain growth while eluding exhaustion from chemotherapy. We therefore tested this hypothesis in APC^{min} adenoma mouse model.

1)

We first established the tumorigenetic kinetics in APC^{min} mouse model with CB57/6 background. We have studied different groups of APC^{min} mice at the ages of 60, 80, 100, 120, 140 days and measured the tumor number and size. The result showed that adenoma in APC^{min} mice withCB57/6 background formed on average at 70-80 days, and mice usually died between 120-140 days. According to the tumorigenetic kinetics, we decided to start our treatment on average 80-90 days when tumor had been established.

2)

Then, we applied local-radiation plus capecitabine (a pro-drug of 5FU) with or without celecoxib (a Cox2 inhibitor) to use the single or combination to treat adenoma in APC^{min} adenoma mouse. Our preliminary result showed that among the different groups as listed above, the combined treatment with 7on/7off was the best to effectively reduce both number and size of tumors in the APC^{min} mice compared to single treatment or vehicle control. Combined treatment can reduce both number and size of tumors in the APC^{min} mice compared to vehicle control as measured by pulse Brdu labeling. We are also monitor hematopoietic system which can be severely damaged by capecitabine. Survival rate of the mice with adenomas that received combined treatment is significantly increased compared to mice that received single treatment.

3)

Thus our novel approach to target cycling CSCs using chemotherapy and quiescent drug-resistant CSCs using Cox2 inhibitor is very efficient to reduce or control adenoma growth. We have established a method to identify chemoresistant cells by combining CIdU (for slow cycling) and IdU (for activation) of drug-resistant cancer cells.

4)

We also established single crypt cell disassociation in solid adenoma, thus facilitating flow assay and purification of cancer stem cells. We identified surface markers CD45, CD44 and CD24 that facilitate enrichment of drug-resistant

cancer cells. Our data showed that $CD24^{lo}Cd44^{hi}$ enriched drug-resistant cancer cells. We have verified that stemness genes are enriched in the sorted $CD24^{lo}CD44^{hi}$ cell population.
5)
Using metastatic markers, MAT1, we observed a significant reduction of metastatic cells following the combination treatment. As radio-chemo mainly eliminated proliferating cells, including active CSCs, and celecoxib inhibited activation of quiescent CSCs via the PGE and β-catenin axis, the combination treatment simultaneously targeted both active and quiescent CSCs, and thus efficiently controlled tumor growth and metastasis.
Therefore, we have established an efficient treatment method based on understanding the cellular and molecular mechanism underlying drug-resistance, and this method will revolutionize the cancer treatment strategy and significantly impact the field of both basic research and clinical treatment of cancers.

F-1041

CHEMOKINE RECEPTOR 4 (CXCR4) HOMING IMMUNOTOXINS TARGETING CANCER STEM CELLS AS BIOMEDICINES FOR METASTATIC CANCERS

Vishal Deshmukh, Sumith Kularatne, Chan Hyuk Kim, Paul Hein, Peter Schultz
Chemistry, The Scripps Res Inst, La Jolla, CA, United States

Despite tremendous progress in the detection and treatment of cancer, tumors often relapse or metastasize and most cancer patients die not from primary tumor burdens, but due to metastatic disease. Radiation and chemotherapy have been widely used but these are primarily designed to kill rapidly dividing cells, thus cause indiscriminate damage to cancer and healthy cells alike, and result in severe side effects. Several targeted therapies aimed at reducing the toxicities of chemotherapeutic agents have been approved or are currently under development but the prognosis remains poor, especially in the treatment of metastatic cancer, and the need for potent and more precisely targeted methods of treating cancer cannot be understated.
The ability of tumors to recur and metastasize remains a major hurdle and there is now increasing evidence to show the primary role of a rare population of cells known as Cancer Stem Cells (CSCs) in tumor development, maintenance, metastasis, and drug resistance. The development of novel cancer therapeutic modalities based on targeting the elimination of CSCs may thus provide a significant advantage in cancer treatment. A number of studies have indicated that chemokine receptor 4 (CXCR4) is over-expressed on CSCs and various metastatic tumors. Further, CXCR4 along with its cognate ligand, SDF1α, participates in homing and mobilization of CSCs into other parts of the body, determining the metastatic destination of tumor cells, thus making the development of CXCR4-targeted therapeutic agents an attractive new approach to fight cancer.
In this context, we designed and developed recombinant human anti-CXCR4 fragment (Fab) and full-length (IgG) antibodies. Further, using a technique for site-specific genetic incorporation of unnatural amino acids (UAAs) we generated mutants of recombinant human anti-CXCR4 fragment (Fab-UAA) and full-length (IgG-UAA) antibodies. These antibodies conjugated to a fluorescent dye (Fab-UAA-AF488 and IgG-UAA-AF488) were able to bind specifically to the cell surface CXCR4 receptor with a high affinity and internalize the dye into human metastatic cancer cells (secondary osteosarcomas collected from lungs), as determined using flow cytometry and confocal microscopy. Further, the mutant antibodies site-specifically conjugated to Auristatin, a toxic chemotherapeutic agent (Fab-UAA-Aur and IgG-UAA-Aur) were able to effectively kill CXCR4 positive tumor cells in vitro (EC50 ~1nM), with negligible toxicity on various CXCR4 negative cell lines. We further evaluated the in vivo efficacy of these CXCR4-targeted anticancer biotherapeutics in an experimental mouse model for metastatic lung cancer. Both Fab-UAA-Aur and IgG-UAA-Aur were able to eliminate established metastatic lung tumors in mice without recurrence (40-days post treatment). In contrast, untreated lung tumors had a growth doubling time of about 5 days. Histopathological analysis as well as studies evaluating the effects of the antibody-drug conjugates on hematopoietic cells and various other tissues are currently ongoing.
The development of targeted toxin delivery via a CXCR4-specific and high affinity ligand not only establishes the paradigm of selective elimination of CSC populations, but will also enable the design and engineering of unique biomedicines that could specifically target various types of tumors, thus the raising the hope that the next cancer drug to enter the clinic might be a specifically targeted antibody-drug conjugate.

F-1042

AN ALTERNATIVE METHOD FOR IDENTIFYING STEM-LIKE CELLS IN HUMAN ESOPHAGEAL CARCINOMA CELL LINES

Taghreed Almanaa, Michael Geusz, Roudabeh Jamasbi
Bowling Green State University, Bowling Green, OH, United States

Cancer stem cells (CSCs) may play a crucial role in tumorigenesis, chemo-radiotherapy resistance, and the recurrence of cancer. Therefore, isolation and characterization of this cell subpopulation is needed for additional progress in CSCs targeting. This study showed for the first time that an attached-cell Aldefluor method (ACAM) can be used to detect CSCs within cells in monolayer culture. To compare ACAM with the standard Aldefluor method for detecting CSCs, fluorescence-activated cell sorting based on Aldefluor was used to isolate CSC-enriched cells from a human esophageal squamous cell carcinoma (ESCC) cell line, YES-2, to create a new line, YES-2CSC. After three cell passages, significantly more CD44 immunostaining cells remained in YES-2CSC than in the YES-2 line. Curcumin, the active ingredient of the spice turmeric, is reported to selectively inhibit growth of CSCs. As predicted, the YES-2CSC line was more sensitive to curcumin than YES-2. Using ACAM to measure intensity of individual cells, YES-2CSC showed significantly higher average Aldefluor staining than YES-2. Furthermore, YES-2 showed higher staining than the YES-2S line, which had survived a curcumin treatment and therefore had a diminished CSC content. When YES-2 and five additional human ESCC cell lines (KY-5, KY-10, TE-8, YES-1, TE-1) were examined using ACAM the high and low-staining lines were KY-5 and TE-1, respectively, whereas TE-8,YES-1, and KY-10 showed intermediate staining. Because a similar order of staining intensity was observed previously using ALDH1A1 immunocytochemistry with these lines, we conclude that ACAM can also indicate the relative level of CSC content. Tumorspheres are known to be enriched in CSCs, and Aldefluor was able to label cells within spheres made from YES-2CSC. When spheres were allowed to differentiate, proliferating and spreading cells at the sphere edge did not stain with Aldefluor, as predicted. These results show that Aldefluor can detect CSCs in both monolayers and tumorspheres. Moreover, this study introduces a new method for formation and growth of tumorspheres without the growth factor supplements normally used in medium for sphere formation. Spheres formed with this method were also sensitive to 60 µM curcumin. These experiments provide additional evidence that curcumin treatments could be effective in limiting proliferation of CSCs and tumor recurrence.

F-1044

A MICRORNA SWITCH REGULATING ASYMMETRIC DIVISION OF COLON CANCER STEM CELLS

Pengcheng Bu[1], Kai-Yuan Chen[2], Stephen Lipkin[4], Xiling Shen[3]
[A]Biomedical Engineering, [B]Electrical and Computer Engineering, [C]Biomedical Engineering, Electrical Engineering, [1]Cornell University, ITHACA, NY, United States, [2]Medicine, Weill Cornell Medical College, ITHACA, NY, United States

microRNAs play important roles in developmental cell fate decisions, tissue homeostasis and oncogenesis. Recent studies show that microRNAs confer robustness to biological processes in distinct ways relative to proteins. Here, we show that the microRNA miR-34a creates a bimodal switch during cell division that controls the choice of early stage colon cancer stem cell (CCSC) daughters to self-renew or differentiate. Asymmetric distribution of miR-34a during ~20% of CCSC divisions results in daughter cell fate asymmetry. Disruption of this miR-34a switch inhibits asymmetric division and alters the balance between self-renewal and differentiation both in vitro and in vivo. Investigation of miR-34a regulation dynamics revealed a mechanism whereby miR-34a generates a bimodal Notch signal specifying the choice of daughter cell self-renewal versus differentiation. This bimodality is caused by mutual sequestration of miR-34a and Notch1 mRNA, which generates a threshold response. In contrast, the canonical cell fate determinant Numb regulates Notch levels in a continuously graded manner. Overall, this study highlights an important microRNA-regulated mechanism to convert noisy input into binary output for robust decision-making that is distinct from protein-regulated mechanisms.

F-1045

THE ROLE OF XRCC4 IN HUMAN COLON CANCER STEM CELL PROPERTIES AND RADIOSENSITIVITY

Sei Sai[1], Toshifumi Wakai[4], Guillaume Vares[2], Tadashi Kamada[3]
[A]Medical Physics Research Program, Research Center for Charged Particle Therapy, [B]Radiation Effect Mechanisms Research Group, [C]Research Center for Charged Particle Therapy, [1]National Institute of Radiological Sciences, Chiba, Japan, [2]Division of Digestive and General Surgery, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Purpose: To investigate relationship between XRCC4 and cancer stem cell properties and further examine how XRCC4 involved in the radiosensitivity of putative human colon cancer stem cells after X-ray or carbon ion beam.
Methods:
Putative cancer stem cells sorted from HCT116-wild type (WT) and XRCC4 KO cells were treated with or without carbon ion or X-ray irradiation and then colony and spheroid formation assay, FACS analysis, gamma-H2AX foci assay, as well as in vivo tumor formation assay were performed.
Results: FACS analysis showed that the percentage of CD44+ and ESA+ cells was significantly increased in XRCC4 KO cells (6.8%, and 7.2% vs 19.2% and 20%), whereas CD133+ was decreased (3.2% vs 1.6%) compared to HCT116-WT cells. The proportion of CD133+ and CD44+ cells was extremely increased in XRCC4 KO cells compared to HCT116-WT cells after X-ray irradiation. There was no change in proportion of ESA+ cells in HCT116-WT cells, but 10-fold enhancement of ESA+ cells was induced in HCT116-XRCC4 KO cells after X-ray irradiation. The number of colony and spheroid formed from CD133+, CD44+/ESA+ cells were significantly higher compared to that from CD133-, CD44-/ESA- cells in HCT116-XRCC4 KO cells, but extremely decreased compared to HCT116-WT cells. Analysis of cell survival fractions showed that CD133+, CD44+/ESA+ cells sorted from XRCC4 KO cells were predominantly radiosensitized compared to the that from HCT116-WT cells, especially after X-ray irradiation. A much more large number and large-sized gamma-H2AX foci were observed in $CD44^+/ESA^+$ cells sorted from XRCC4 KO cells compared to that from HCT116-WT cells, after 24 h carbon ion beam compared to X-ray irradiation. The in vivo tumorigenicity of XRCC4 KO cells is still retained and there are no differences between CD133+, CD44+/ESA+ cells and CD133-, CD44-/ESA- cells which sorted from XRCC4 KO cells.
Conclusion: In conclusion, lack of XRCC4 significantly altered expression of cancer stem cell markers, radiosensitized cancer stem-like cells to both X-rays and carbon ion berams, suggesting that XRCC4 may play pivotal role in modulating cancer cell stemness.

F-1046

NOTCH SIGNALING IN CANCER STEM CELLS: IMPLICATION IN TUMOR HETEROGENEITY AND THERAPEUTIC TARGETING

Ankur Sharma, Annapoorni Rangarajan, Rajan R. Dighe
Molecular Reproduction Development and Genetics, Indian Institute of Science, Bangalore, India

Notch signaling pathway plays a key role in regulating various cell fates including those of stem cells, throughout the mammalian development and adult homeostasis. Here we report characterization of anti-Notch1 monoclonal antibodies (MAb's) with potential therapeutic applications. T-Acute Lymphoblastic Leukemia (T-ALL) patients were

screened for mutation in Notch1 Negative Regulatory Region (NRR) and murine MAb's specific to these pathogenic mutations were generated. MAb's against mutant form of Notch1 receptor were characterized in various solid phase and cell based assays. The conformation specific MAb's against Notch1 can specifically distinguish between the mutant and the wild type receptors present on cancer cells. Anti-NRR MAb's 604.107 (mutant specific) and 604.132 (wild type specific) specifically inhibited Notch1 signaling and proliferation in breast, colon and leukemia cell lines and primary cells. MAb 604.107 inhibited sphere forming efficiency in breast and colon cancer cells, depleted CD 44high/CD24Low and CD34High/CD45High sub-populations in breast cancer and T-ALL cells respectively. Further, MAb 604.107 treatment leads to apoptosis, inhibition of epithelial-to-mesenchymal transitions as well as in vivo tumor formation in mouse model. Antibody treatment further improved the efficacy of chemotherapeutic agents by inhibiting expression of ABCC1 transporter. These MAbs can serve as a potential tool to irreversibly combat therapy resistant cancer stem cell sub-populations and to inhibit the epithelial to mesenchymal transitions in solid tumors and hematological malignancies.

Chromatin in Stem Cells

F-1051

MECHANISMS BY WHICH ARID3A FACILITATES REPROGRAMMING

Troy D. Templeton[1], Robert Barstead[2], Graham Wiley[2], Patrick M. Gaffney[2], **Carol F. Webb**[1]
[A]Immunobiology and Cancer, [B]Arthritis and Clinical Immunology, [1]Oklahoma Medical Research Foundation, Oklahoma City, OK, United States

We previously showed that inhibition of the DNA-binding protein, ARID3a, in human 293T cells resulted in increased expression of Klf4, Oct4, Sox2 and c-myc. Furthermore, these cells, and adult cells from ARID3a deficient mice, formed phenotypically altered colonies that had the capacity to express markers of all three germ line lineages. These results revealed an association between ARID3a inhibition and formation of developmentally plastic cells, suggesting that ARID3a might play a role in reprogramming. We recently observed that ARID3a deficient mouse embryonic fibroblasts spontaneously form colonies that resemble induced pluripotent stem cells, and found that the Oct4 promoter in these cells was hypomethylated compared to mouse ES controls. ChIP-seq data identified a number of pluripotency related genes that may be directly regulated by ARID3a, including Oct4. We found that the human Oct 4 promoter is bound by ARID3a in human primary fibroblasts. Inhibition of ARID3a with shRNAs in these cells also resulted in formation of developmentally plastic cells similar to those we observed in ARID3a deficient mouse cells. We hypothesize that ARID3a may act to suppress Oct4 transcription in adult differentiated cells and that its inhibition facilitates the reprogramming process by initiating events that allow Oct4 expression. Analyses of ARID3a-inhibited fibroblasts suggest that global changes in H3 histone modifications are initiated within a few days of ARID3a silencing. Preliminary data indicate that some of these changes occur at the Oct4 promoter; active histone marks appear to increase, while repressive marks decrease in response to ARID3a inhibition. Together, these data suggest previously unappreciated roles for ARID3a in the reprogramming process.

F-1052

VARIATIONS IN CHROMOSOME TERRITORY INTERACTIONS WITH DIFFERENTIATION

Scott Davidson, Neil Macpherson, Jennifer A. Mitchell
Department of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada

Chromatin within the nucleus of embryonic stem (ES) cells has distinct characteristics compared to other cell types. It has been demonstrated that chromatin within ES cells is less condensed than in differentiated cells; with more dynamic heterochromatin proteins present displaying increased mobility. Furthermore, bivalent domains composed of both active and inactive histone methylation marks are found at genes poised for transcription upon differentiation. In addition, studies of nuclear ultrastructure by EM have revealed an absence of condensed chromocenters within ES cell nuclei. It has also been noted that ES cells display higher transcriptional activity along with an increased number of transcription factories.
Microscopy methods in other cell types have shown chromosome territories to have intermingling domains that contain transcription factories allowing genes from different chromosomes to co-occupy factories. Genome-scale variants of the chromosome conformation capture technique, such as Hi-C, have provided molecular based data on chromatin intermingling, however the interactions between different chromosomes have been under investigated. By interrogating Hi-C data we observed that interactions between different chromosomes can account for 50% of a sequenced Hi-C library. Furthermore, both human and mouse ES cells display substantially fewer (10-15%) inter-chromosomal interactions than differentiated cell types. In all cell types investigated common and unique chromosome paring preferences were observed in the Hi-C data. Chromosome pairs were chosen based on the Hi-C data and investigated by DNA FISH. Pairs chosen for their close nuclear proximity based upon Hi-C interaction data were used to ascertain that the chromosome territories of these pairs within ES cells are significantly further apart than they are within differentiated cells.
Our study highlights the conformity between the molecular data of Hi-C studies with that of fluorescence based microscopy studies at the scale of chromosome territories. Intriguingly our study has also revealed that individual chromosome territories intermingle to a lesser degree in ES cells which may play an important role in maintaining the pluripotent phenotype.

F-1053

PREDICTION OF CHROMATIN STATE VARIABILITY

Luca Pinello[1], Jian Xu[2], Stuart H. Orkin[2], Guocheng Yuan[1]
[1]Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA, United States, [2]Division of Hematology/Oncology, Children's Hospital Boston, Boston, MA, United States

Chromatin states are highly cell-type specific, and the underlying regulatory mechanism remains poorly understood. In this study we have developed a computational approach to systematically identify potential regulatory factors by integrating information from multiple cell lines. We quantified the overall variability of a chromatin mark associated with each genomic locus, and focused on analyzing the most variable regions (MVRs) in order to gain insights into cell-type specific regulation. We chose the H3k27me3 mark as an example, because it is well-known to play an important role in developmental control. The MVRs are generally associated with CpG islands, but a significant fraction falls into distal regions. H3K27me3 occupancy at these distal MVRs tends to be highly cell-type specific, in that it is only present in a small number of cell-types. The locations of the MVRs can be well-predicted from the DNA sequence information. However, the sequence features associated with distal MVRs are distinct from those in CpG islands and highly enriched with cell-type specific transcription factor binding motifs. Using this approach, we analyzed our recently generated ChIPseq dataset in human stem/progenitor cells (HSPC) in search for potential Polycomb recruiting factors. Surprisingly, we found that the sequence motif of TAL1, which is commonly known as an activator, is strongly associated with HSPC specific MVRs. This association was further validated by follow-up ChIP-seq experiments. In addition, we found that the Polycomb recruitment activity is mediated by cofactor GFI1B. Thus, our analysis has led to the discovery of a previously unrecognized repression role of TAL1 and its genomic context. This approach is generally applicable to analysis of any other epigenetic mark or their combinations.

F-1054

HISTONE LYSINE METHYLTRANSFERASE SUV4-20H1 IS REQUIRED FOR MUSCLE STEM CELL QUIESCENCE AND MUSCLE INTERGETY

Verawan Boonsanay, Yonggang Zhou, Thomas Braun
Cardiac Development and Remodelling, Max Planck Institute for Heart and Lung Research, Bad nauheim, Germany

Stem cells can be distinguished from committed cells by distinct chromatin configuration and epigenetic signature. For example a rare stem cell population termed satellite cell (SC) in adult skeletal muscle shows a condensed chromatin organization whereas fully differentiated myocyte harbors more open chromatin. However the epigenetic mechanisms that are responsible for maintaining such condensed chromatin structure in SCs hence the muscle stem cell identity *in vivo* are still elusive. Here we show that condensed chromatin in SCs is facultative heterochromatin with combined enrichment of H3K9me3 and H3K27me3 whereas fully differentiated myocytes harbor more constitutive heterochromatin with enriched modifications of H3K9me3 and H4K20me3. Interestingly transition from mono and dimethyl H4K20 to trimethyl H4K20 coordinates with muscle stem cell differentiation. Consistently conditional ablation of Suv4-20h1, the enzyme catalyzes histone H4K20me2, in Pax7-derirved muscle precursors results in lost of facultative heterochromatin characteristics in quiescent SCs. This leads to SC activation and hyperproliferation. Consequently muscles lacking Suv4-20h1 expression show muscle hyperplasia without increased myofiber size and delayed muscle differentiation upon acute muscle injury. The perturbation of SC quiescence upon Suv4-20h1 ablation can be partially explained by the result that Suv4-20h1, together with SRF, suppresses master muscle regulator *MyoD* gene expression in quiescent SCs. These findings reveal a novel epigenetic regulatory pathway consisting of SRF and Suv4-20h1 in control of facultative chromatin formation, muscle stem cell homeostasis and muscle integrity.

F-1055

THE NANOG LOCUS ENGAGES IN A PLURIPOTENCY-SPECIFIC, GENOME-WIDE INTERACTION NETWORK THAT CHANGES UPON DIFFERENTIATION AND IS RESET IN IPS CELLS.

Effie Apostolou[1], **Francesco Ferrari**[2], Ryan Walsh[1], Ori Bar-Nur[1], Matthias Stadtfeld[3], Sihem Cheloufi[1], Peter V. Kharchenko[2], Peter J. Park[2], Konrad Hochedlinger[1]

[1]Cancer Center and Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA, United States, [2]Center for Biomedical Informatics, Harvard Medical School, Boston, MA, United States, [3]Kimmel Center for Biology and Medicine, The Skirball Institute of Biomolecular Medicine, New York University, New York, NY, United States

Pluripotency is maintained by a number of key genes whose activity is governed by specific molecular mechanisms, such as the binding of transcription factors, deposition of epigenetic marks and the looping between promoter and enhancer elements. However, it remains unclear whether pluripotency loci also undergo broader, cell type-specific changes in three-dimensional chromatin organization in the course of differentiation and cellular reprogramming into induced pluripotent stem cells (iPSCs).

Here, we utilized a modified circular chromosome conformation capture-sequencing (m4C-seq) method, based on Ligation Mediated-PCR of biotinilated primer recognizing the Nanog locus. Specific enrichment and purification of the Nanog-interacting regions is achieved with streptavidin beads. Thus we avoid the less efficient re-circularization and inverse-PCR steps, which are required in previously published 4C methods.

We identified a genome-wide network of intra- and inter-chromosomal interactions involving the Nanog promoter in embryonic stem cells (ESCs), differentiated cells, and iPSCs. We found a pluripotency-specific pattern of interactions that is rearranged after differentiation and restored upon somatic cell reprogramming. We further found that more than 40% of Nanog-interacting loci involved components of the mediator or cohesin complex in pluripotent cells. Depletion of those proteins from ESCs resulted in a selective disruption of contacts and the acquisition of interaction patterns of differentiated cells.

Our results document a complex, pluripotency-specific chromatin interactome for Nanog and suggest a functional role for long-range genomic interactions in the induction and maintenance of pluripotency.

F-1056

WIDESPREAD CHROMATIN REMODELING DURING HUMAN ENDOTHELIAL CELL REPROGRAMMING

Ekaterina M. Vassina[1], Aleksey S. Sokolov[2], Svetlana V. Zhenilo[2], Alexandra N. Bogomazova[1], Alexander Mazur[2], Maria Shutova[1], Egor B. Prokhorchuk[2], Sergey L. Kiselev[1], Maria A. Lagarkova[1]

[1]*Stem Cell Laboratory, Vavilov Institute of General Genetics, Russian Academy of Science, Moscow, Russian Federation,* [2]*Centre Bioengineering, Russian Academy of Sciences, Moscow, Russian Federation*

Cell reprogramming is a developmental reversal program that involves gradual loss of cell-type specific features and acquisition of pluripotent cell state. To gain further insight into the mechanisms of chromatin-based and transcriptional reprogramming events we performed analysis of genome wide epigenetic and transcriptional profiles of initial somatic human umbilical vein endothelial cell (HUVEC) and resulting induced pluripotent cell populations. HUVECs with the virtual absence of cell mutagenic events have been shown to be safer source of iPS cells and thus serve as an excellent model of reprogramming process. During this study we generated genome-wide chromatin maps of H3K4me2 histone modification as the general mark of both promoter and enhancer regions, and H3K27me3 as the silencing mark in particular associated with developmental genes. To investigate cell type specific histone methylation profiles we employed tactic for identification of ChIP-enriched marks in various regions of gene and parts of intergenic regions. This approach revealed the differential organization of H3K27me3-enriched regions in H3K27me3-only transcripts compare to 'bivalent domains'. Overall, we identified more than 20000 and 9500 H3K4me2-only transcripts in HUVEC and iPS cells correspondently, which contained activation mark in at least one gene region. Silencing of most of lineage-specific genes, including majority of transcription factors were accompanied to a large extent by disappearance of H3K4me-only signature, without H3k27me3 gain and involved 55% of somatic transcripts. Another 45% of H3K4me-only enriched transcripts either did not change significantly or acquired enhanced mark during reprogramming and contributed to more than 80% of active H3K4me-enriched transcripts in pluripotent cells. Our integrated data analysis will help to reveal the complex machinery that defines cell identity.

F-1057

HIERARCHICAL ANNOTATION OF CHROMATIN STATES IN STEM CELLS

Eugenio Marco, Luca Pinello, Guo-Cheng Yuan
Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA, United States

Epigenetic mechanisms play an important role in stem cell processes, but our mechanistic understanding of epigenetic regulation is still incomplete. One major difficulty is that chromatin forms complex three-dimensional structures and studies have failed to map genome-wide chromatin interactions with enough resolution. On the other hand, genome-wide distributions of the first-order chromatin structure, such as histone modifications, have been characterized at increasingly higher resolution. In order to identify multi-layer chromatin structures simultaneously, we have developed a Hierarchical Hidden Markov Model (HHMM) with two-layers of chromatin states, which we call domain- and bin-level states, respectively. Using this method, we analyzed a ChIPseq dataset of 9 histone marks in human embryonic stem cells, and identified a number of chromatin domains that can be validated by independent studies. At the same time, bin-level states detected variations in histone modification patterns at high resolution. Our new HHMM approach has uncovered higher order chromatin states and provides novel insights into stem cell regulation.

F-1058

DYNAMICS OF X-CHROMOSOME INACTIVATION IN HUMAN EMBRYOS AND NEWLY DERIVED HUMAN PLURIPOTENT STEM CELLS

Shila Mekhoubad[1], dieter egli[2], alex meissner[1], Kun Zhang[3], kevin eggan[1]
[1]*Harvard University, Cambridge, MA, United States,* [2]*New York Stem Cell Foundation, New York, NY, United States,* [3]*University of California, San Diego, La Jolla, CA, United States*

In female mammals, the increased dosage of X-linked genes is compensated for by transcriptional inactivation of one of the two X chromosomes. This process of X-chromosome inactivation (XCI) takes place in the early embryo

resulting in random choice of one X for chromosome-wide gene silencing. In the female mouse, the pluripotent state of the inner cell mass (ICM) of the blastocyst and established embryonic stem cells (ESC) is tightly coupled with two active X chromosomes. Upon differentiation, one of the two X chromosomes is randomly inactivated. In comparison to the mouse, little is known about the developmental regulation of X-chromosome inactivation (XCI) in humans. Previous reports in human embryos and derived ESCs have produced variable results. Here, we used RNA FISH and immunostaining for the hallmarks of the inactive X-chromosome to show that unlike in the mouse, cells within the inner cell mass (ICM) of female, but not male, human blastocysts contain repressed chromatin indicative of an inactive X chromosome. Consistent with the notion that human ESC lines are derived from cells of the human ICM that are either undergoing, or have already undergone XCI, we found that the very first colonies of ten newly derived female human ESC lines were uniformly composed of cells containing an inactive X chromosome. We further found that modifying the atmospheric conditions did not lead to the derivation of lines in their pre-X inactivation state regardless of weather embryo culture and human stem cell derivation were carried out under ambient or physiological oxygen conditions. However, as been previously reported, during routine culture, we observed that human pluripotent stem cells gradually lost cytological signs of their inactive X chromosome, including histone methylation and coating with XIST RNA. These marks were irreversibly lost and were not upregulated upon cell differentiation. Strikingly, we found that oxygen conditions had a significant modifying influence on the rate at which these marks were lost with lines grown at low oxygen conditions losing XCI marks much more rapidly than the same lines grown at high oxygen. Further methylaion and allele specific analysis of X-linked genes in isogenic lines with and without XCI marks revealed that up to 40% of genes on the X-chromosome were subjected to reactivation from the former inactive X chromosome in lines that have lost XCI marks. In summary, our results suggest that the native state of human ESC is to have an inactive X chromosome. Epigenetic instability during prolonged culture and varying levels oxygen exposure, however, lead to loss of XCI marks and irreversible reactivation of X linked genes. Our findings here illuminate an important developmental difference between humans and mice, while having important implications for the use of female pluripotent stem cells for disease modeling or other translational studies.

F-1061

RESPONSIVENESS TO NEURAL INDUCING COMPOUNDS BY RAT PLURIPOTENT STEM CELLS

James Hong[1], Peter McDonald[2], Mark Weiss[1]

[1]Kansas State University, Manhattan, KS, United States, [2]University of Kansas, Lawrence, KS, United States

There is extensive literature with respect to neural differentiation of human and mouse pluripotent stem cells (PSCs). Human and mouse PSCs have been differentiated to various neural progenitors capable of differentiating to neurons and glia, including primitive neuroectoderm, neuroepithelial, radial glia and definitive neural stem cells. The neuroectodermal and neuroepithelial cell types are representative of neural plate and neural tube, respectively. In contrast, little has been published about differentiation of rat PSCs to neural fates. Here, we tried several different protocols for the induction of rat PSCs to the neuroectoderm and neuroepithelial state including dual SMAD inhibition, basic fibroblast growth factor (Fgf2) and stromal cell co-culture. We found that both SMAD2 inhibition and GSK inhibition in combination with LIF and Fgf2 can induce nestin-positive neural stem cell-like derivatives which can be further differentiated to Map2 and Tubb3 expressing cells. We examined different rat PSC lines and found cell line-specific differences in responsiveness to these compounds. In addition, we found that plating density affects the proliferation rate of these neuroepithelial-like rat PSC derivatives. In summary, we have designed a protocol for the consistent derivation of a neural precursor-like cell from rat PSCs.

Pancreatic Cells

F-1063

COLONY FORMING CELLS IN MOUSE POSTNATAL LIVER AND PANCREAS GIVE RISE TO MORPHOLOGICALLY DISTINCT INSULIN-EXPRESSING COLONIES IN THREE-DIMENSIONAL CULTURES

Liang Jin[1], Tao Feng[1], Jing Chai[1], Jasper Hsu[1], Alborz Mahdavi[2], David A Tirrell[2], Arthur D Riggs[1], Hsun Teresa Ku[1]
[1]City of Hope, Beckman Research Institute, Duarte, CA, United States, [2]California Institute of Technology, Pasadena, CA, United States

In our previous studies, colony-forming cells isolated from murine embryonic stem cell-derived cultures were differentiated into morphologically distinct insulin-expressing colonies that were small and not light-reflective (named "Dark" colonies) when observed by phase-contrast microscopy. A single cell capable of giving rise to a Dark colony was termed a Dark colony-forming unit (CFU-Dark). The goal of the current study was to test whether the pancreas, and its developmentally related liver, harbored CFUs-Dark. Here we show that dissociated single cells from liver and pancreas of one-week-old but not adult mice give rise to Dark colonies in semisolid culture media containing either Matrigel or laminin hydrogel (an artificial extracellular matrix protein containing elastin and alpha1 laminin sequences). CFUs-Dark comprise approximately 0.1% and 0.03% of the postnatal liver and pancreas, respectively. Microfluidic qRT-PCR analysis of individually handpicked colonies reveals the expression of insulin genes in many but not all Dark colonies. Most pancreatic insulin-positive Dark colonies also express glucagon, whereas liver colonies do not. Liver CFUs-Dark require Matrigel but not laminin hydrogel to become insulin-positive. In contrast, laminin hydrogel is sufficient to support the development of pancreatic Dark colonies that express insulin. Liver CFUs-Dark display a cell surface marker $CD133^{+}CD49f^{low}CD107b^{low}$ phenotype, while pancreatic CFUs-Dark are $CD133^{-}$. Together, these results demonstrate that progenitor-like cells in the postnatal liver and pancreas are capable of developing into insulin-expressing colonies, but they differ in frequency, marker expression and matrix protein requirements for growth.

F-1064

THE ROLE OF MICRORNA IN THE DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS INTO DEFINITIVE ENDODERM

Natalie Francis[1], Melanie Moore[1], Guy A. Rutter[2], Christopher J. Burns[1]
[1]Biotherapeutics, NIBSC, Potters Bar, United Kingdom, [2]Department Of Medicine, Imperial College London, London, United Kingdom

Type 1 diabetes mellitus (T1DM) results from auto-immune destruction of pancreatic ß cells. Pancreatic islet transplantation has shown proof of principle for cell replacement therapy to treat T1DM. Insulin-expressing cells have been produced in vitro from embryonic stem cells (ESCs), however problems associated with ESCs, primarily ethical concerns and immunogenicity, mean an alternative cell source is needed.
Induced pluripotent stem cells (iPSCs) are an alternative source of pluripotent stem cells and can be derived in a patient-specific manner. iPSCs have been shown to differentiate into insulin-expressing cells in vitro but it is unknown whether iPSCs are equivalent to ESCs, since important differences in gene expression, DNA methylation & microRNA expression have been shown to exist between ESCs & iPSCs. These differences may affect the ability of iPSCs to give rise to cells of a pancreatic lineage and thus limit their usefulness for a clinical application.
MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally. They are differentially expressed depending on cell type and stage of differentiation. Some miRNAs are only expressed in pluripotent stem cells, although iPSCs often have lower levels of these than ESCs. MiRNAs also play specific roles in development and a unique miRNA signature characterises early pancreas development at the definitive endoderm (DE) stage. However, since there are differences in miRNA expression between iPSCs & ESCs, this may affect their ability to undergo directed differentiation when compared to ESCs.

Results: Multiple iPSC & ESC lines were differentiated into DE using an Activin A-based protocol. Individual cell lines showed different propensities to form DE so microarray analysis was carried out on undifferentiated and differentiated iPSCs & ESCs in order to compare miRNA expression.
Microarray analysis allowed the identification of miRNAs that are up- or down-regulated upon differentiation to DE and which may play a role in this process. The 10 miRNAs that are most upregulated in DE are miR-375, miR-708-5p, miR-744-5p, miR-4792, miR-4530, miR-26b-5p, miR-4472, miR-27b-3p, miR-4289 & miR-30b-5p. The 10 miRNAs that are most downregulated on DE formation are: miR-5002-5p, miR-378a-3p, miR-3941, miR-4451, miR-516b-5p, miR-4436b-5p, miR-4732-3p, miR-32-3p, miR-124-5p & miR-4285. Several of these miRNAs have been previously implicated in pancreas development.
Comparison of undifferentiated iPSCs & ESCs showed that they share a miRNA signature, with no miRNAs differentially expressed ($p>0.05$). However, upon differentiation 91 miRNAs were differentially expressed between ESCs & iPSCs: 61 were downregulated in iPSCs vs. ESCs, and 30 were up-regulated. This suggests that miRNA expression may play an important role in the ability of iPSCs & ESCs to differentiate into DE.
Microarray results have subsequently been validated by qRT-PCR. We are now investigating the role of miRNAs that are involved in DE formation and are differentially expressed between ESCs & iPSCs through the identification of putative targets and the manipulation of miRNA expression in differentiating cells.
Summary sentence: iPSCs are a promising alternative to ESCs for the treatment of T1DM. However, differences in miRNA expression between these two cell types may affect their ability to differentiate into the pancreatic lineage, which may be important for any future clinical application of these cells.

F-1065

SCREENING OF SMALL COMPOUNDS TO PROMOTE DIFFERENTIATION OF MOUSE ES CELLS TO FUNCTIONAL PANCREATIC β CELLS.

Daisuke Sakano[1], Nobuaki Shiraki[1], Kazuhide Kikawa[1], Fumio Endo[1], Kazuhiko Kume[1], Motonari Uesugi[2], Shoen Kume[1]

[1]Kumamoto University, Kumamoto, Japan, [2]Kyoto University, Kyoto, Japan

Islet transplantation has been suggested to be a promising treatment for type 1 diabetes, and embryonic stem (ES) and induced pluripotent stem (iPS) cells are potentially unlimited cell sources of transplantable islet cells. Therefore, the establishment of new approaches to induce ES cells or iPS cells to differentiate into insulin-producing cells will contribute to transplantation therapy in diabetes.
We previously reported that mouse ES cell is efficiently differentiated into pancreatic progenitor cells when they are cultured on M15 cells and added with growth factors (Shiraki *et al*., 2008). We also reported that M15 cell secretes **extracellular** matrices, which potentiate ES cells to differentiate into Pdx1-expressing pancreatic progenitor cells. The extracellular matrices can be reconstituted by a synthetic basement membrane (sBM) (Higuchi *et al*., 2010). We transplanted the cells grown on sBM into kidney
capsule of SCID mice to evaluate differentiation into pancreatic b-cells. One month after transplantation, many insulin-positive cells made clusters in the recovered graft.
These results suggested that extracellular matrix and/or suitable scaffolds would potentiate the differentiation of ES cells into insulin producing cells. Then we found a three-dimensional scaffolds stably promoted the differentiation from ES cells to Pdx1 positive pancreatic progenitor cells. Approximately 0.1% of cells could be insulin producing cells when we continued the cultured on the three-dimensional scaffolds.
In an attempt to establish a high throughput screening assay, this system is useful to seek small compounds that promote pancreatic beta cell differentiation. Then, we have screened more than 1300 compounds and identified 10 compounds that could increase β-cell number.
The chemical library which we used consisted of pharmacologically identified bioactive small compounds. We classified the hit compounds into several groups according to their target cascades. Now we are confirming the effect of the compounds, including using shRNA strategies. The use of compounds that regulate different target molecules and/or pathways is a useful tool and the effects combining these compounds can be studied. The cells con-

tinue to produce insulin *in vivo* after transplanting to mice under kidney capsules and the effects on lowering the blood sugar level were studied.

F-1066

FACTORS EXPRESSED BY MURINE EMBRYONIC PANCREATIC MESENCHYME ENHANCE GENERATION OF INSULIN-PRODUCING CELLS FROM HESCS

Tingxia Guo, Limor Landsman, Na Li, Matthias Hebrok
Diabetes Center, Univ of California, San Francisco, San Francisco, CA, United States

Islet transplantation has proven to be a successful strategy to restore normoglycemia in type 1 diabetes (T1D) patients. However, the dearth of cadaveric islets available for transplantation hampers the widespread application of this treatment option. Our goal is to identify embryonic signaling molecules or tissues that induce development of functional insulin-producing cells from stem cells. Previous studies have shown that hES/iPS cells can be differentiated into pancreatic progenitor cells, which have the ability to mature into functional beta cells when transplanted into mice or rats. However, the signaling molecules that can induce the maturation of endocrine progenitors have yet to be identified. Pancreatic endoderm interacts with surrounding tissues, such as pancreatic mesenchyme, and soluble factors secreted from pancreatic mesenchyme, like FGF10, have been shown to induce the development of pancreatic progenitors. We have identified factors secreted by the mesenchyme during pancreatic development in mouse embryos through differential gene expression arrays. Candidate factors have being added alone or in combination to test their function on pancreatic progenitors and on beta cell maturation in vitro. Several of the identified factors work in concert to expand the pancreatic progenitor pool. Interestingly, TGF-ß ligands, most potent in inducing pancreatic progenitors, display strong inhibitory effects on subsequent endocrine cell differentiation. Treatment with TGF-ß ligands followed by addition of a TGF-ß receptor antagonist dramatically increased the number of insulin-producing cells in vitro, demonstrating the need for dynamic temporal regulation of TGF-ß signaling during in vitro differentiation. These studies illustrate the need to precisely mimic the in vivo conditions to fully recapitulate pancreatic lineage specification in vitro.

F-1067

CO-TRANSPLANTATION OF ISLETS WITH HUMAN MESENCHYMAL STROMAL CELLS

Telford Yeung, Deb Dixon, Karen Seeberger, Adetola Adesida, Tatsuya Kin, Gregory Korbutt
University of Alberta, Edmonton, AB, Canada

Objective:
Islet transplantation offers metabolic benefits for patients with type 1 diabetes, but recipients progressively lose insulin independence as a result of inflammation mediated graft dysfunction. We previously demonstrated that mesenchymal stem cells (MSCs) could protect human islets from pro-inflammatory cytokines (IL-1β, TNF-α, and IFN-γ) *in vitro*. Others have demonstrated that MSCs can improve mouse islet function after transplantation. Thus in this study, we hypothesized that MSCs could improve islet transplant outcomes in a mouse model of diabetes with clinically relevant islet sources. We utilized human islets, the main tissue source for clinical islet transplantation, and xenogeneic neonatal porcine islets (NPI), an abundant and promising alternative source of islets.
Methods:
To examine the *in vivo* effects of MSCs, adult human or neonatal porcine islets were co-cultured with human MSCs and transplanted into the renal subcapsular space of diabetic immunodeficient mice. Weekly glycemic levels were measured and upon reversal of diabetes, the engrafted kidney was removed to confirm the function of the graft.
Results:
After human islet transplantation, average weekly glycemia in the islet group were lower than the islet:MSC group. Also, a greater proportion of the islet group achieved euglycemia compared to the islet:MSC co-transplant group (87.5% vs. 25.0%, n=8, $p<0.01$). However, after neonatal porcine islet (NPI) transplantation, a greater proportion of the co-transplant group achieved euglycemia compared to the NPI group (75.0% vs. 25.0%, n=4 mice per group).

Discrete aggregates of insulin positive cells surrounded by collagen fibrils were present in the co-transplant group; whereas limited organization of insulin positive cells and abundant amounts of collagen deposits were evident in the NPI group.

Conclusions:

Co-transplantation of MSCs with human islets did not improve islet graft function, as the increase in graft size with MSCs likely delayed engraftment, leading to islet loss. An alternative site could be the omental pouch, which can accommodate larger grafts. In contrast, NPI engraftment and function was better in the NPI:MSC co-transplant group compared to the NPI only group. This study demonstrates that MSCs may facilitate neonatal islet engraftment, but considerations regarding transplant site are important for successful engraftment of adult human islets.

F-1068

ENHANCED MAINTENANCE OF RAT PANCREATIC ISLET OF LANGERHNAS ON LAMININ-COATED ELECTROSPUN NANO MATRIX IN VITRO

Mozhdeh Sojoodi, Ali Farrokhi, Azadeh Moradmand, Hossein Baharvand
Royan Institue, Tehran, Iran, Islamic Republic of

Introduction: To achieve successful pancreatic islet transplantation, it is important to optimize the in vitro culture environment and keep the islets viable and functional. The purpose of this study was investigating the effect of nanofiber matrix combination with laminin, on rat islets of langerhans adhesion, morphology, viability and functionality after 7 days in vitro culture.

Materials and Methods: Adult male rat islets were seeded on dishes coated with laminin in combination with Ultra web nanofiber matrix. BSA coated dish considered as a control. Morphological investigation was preformed 72-h post islets seeding and Annexin-V-FITC apoptosis detection Kit was used for determination of viable, necrotic and apoptotic cell population 7 days post seeding. Moreover, insulin secretion was assessed by the glucose challenge test (3.3 mM glucose and 16.7 mM glucose) and islet specific genes expression (Ins1, Ins2, Gcg, Glut2, Nkx6.1) was evaluated by qRT-PCR.

Results: The number of islets attached to nanofiber coated with laminin was significantly higher in comparison to other groups. Moreover, the islets attached firmly to nanofiber coated with laminin with no shape alteration and further and less necrotic area. Islets cultured on this group showed significant increase in mRNA expression of Ins1, Ins2 among other groups ($n=3$, $P<0.05$). Furthermore, just islets on nanofiber coated with laminin dishes were responsive to glucose stimulation after7 days ($n=3$, $P<0.05$).

In according to mentioned results, Nano fiber Matrices can provide suitable ECM environment to reach optimum attachment and cell viability for islets culture. This study provides a basis for the future establishment rat pancreatic islet of langerhans culture with optimum condition.

F-1071

CONTINUOUS REMODELING AND TISSUE INTEGRATION OF IN VIVO INDUCED PANCREATIC BETA CELLS WITH LITTLE CELL PROLIFERATION

Qiao Zhou[1], Weida Li[1], Claudia Cavelti-Weder[2], Yingying Zhang[1], Kendell Clement[1], Shaun Mahony[3], Alexander Meissner[1], Gordon Weir[2], David Gifford[4]
[A]Stem Cell and Regenerative Biology, [B]Joslin Diabetes Center, [1]Harvard University, Cambridge, MA, United States, [2]Pennsylvenia State University, Pittsburg, PA, United States, [3]Massachusettes Institute of Technology, Cambridge, MA, United States

Direct lineage conversion has emerged in recent years as an important component of regenerative medicine. Many therapeutically important cell types can now be generated directly by lineage reprogramming from heterologous cells: fibroblasts to neurons or cardiomyocyte, pancreatic acinar cells to beta cells, among others. A central question of the lineage reprogramming field is how closely the induced cells resemble their endogenous counterparts

and therefore can be used to model endogenous function. For the purpose of cell replacement therapy, it is also critical to evaluate how well the induced cells can integrate into an existing adult organ.
Our laboratory previously developed an in vivo reprogramming model whereby pancreatic acinar cells are directly converted to insulin-secreting beta cells by the coordinated activity of three transcription factors. We now demonstrate that although Insulin+ beta-like cells arise rapidly upon reprogramming factor expression, these cells lack proper glucose-regulated insulin secretion. The induced beta cells only attain key physiological properties after a prolonged period in vivo, up to 2 months. RNA profiling and global DNA methylation analyses revealed extensive and continuous remodeling of the induced cells over a prolonged period. Surprisingly the large-scale changes we observed occur without significant cell proliferation. The overall turnover rate of the induced beta cells is similar to endogenous beta cells (0.12% per day from 3 month to 6 month). Large numbers of epigenetic modifying factors and histone proteins are activated during the reprogramming process, which may underlie these intracellular changes. Our studies further show that the induced beta cells, although initially scatted throughout the exocrine pancreas, aggregate into islet-like structures over time. The induced islets recruit vascular support and neuronal innervation. Some of the induced islets fuse with endogenous islets to form hybrid structures. Our studies demonstrate that lineage reprogrammed cells undergo continuous and extensive transcriptional and epigenetic changes without significant cell proliferation. We confirmed that induced cells can attain refined physiological functions and fully integrate into a preexisting adult organ.

F-1073

EFFECTS OF PDX-1 EXPRESSION CONTROL ON THE DIFFERENTIATION FROM MOUSE ES CELLS INTO INSULIN-PRODUCING CELLS

Mikako Saito, Nobuaki Hanata, Yukari Kakutani, Hideaki Matsuoka
Tokyo Univ of Agriculture & Technology, Tokyo, Japan

One promising approach for the development of cell therapies for diabetes treatment is to utilize pancreatic endocrine cells differentiated from pluripotent stem cells such as embryonic stem (ES) cells. Our group has been developed an original method to induce the differentiation into insulin-producing cells from mouse ES cells (mES cells). However, the insulin secretion ability of the differentiated cells was not enough to improve blood glucose *in vivo*, suggesting that the cells we obtained were not fully functional mature [[Unsupported Character - Symbol Font ]] cells. *Pdx-1*, a homeodomain transcription factor, has a crucial role for regulation of pancreatic development and characteristic expression pattern. Our group has established a mouse ES cell line which is capable of controlling exogenous *Pdx-1* expression by means of Tet-off system integrated into the Rosa-locus. Therefore, in this study, we examined the effects of *Pdx-1* expression control during the differentiation process into insulin-producing cells by using this cell line, and our differentiation protocol for the purpose of generating functional insulin-producing cells. The results showed that induced overexpression of exogenous *Pdx-1* during the differentiation process upregulated the mRNA expression of insulin (*Ins1* and *Ins2*) and other pancreas-related genes. Insulin secretion analysis also revealed that insulin protein was highly produced in the differentiated cells with overexpression of exogenous *Pdx-1*. Furthermore, temporal overexpression of exogenous *Pdx-1* during the differentiation process enhanced the mRNA expression of insulin and the differentiated cells secreted insulin protein in response to KCl, although they did not response to glucose. These results suggested the importance of regulating *Pdx-1* expression during differentiation, rather than overexpressing it continuously. Taken together, the present study showed the utility of regulating exogenous *Pdx-1* expression in a temporal manner as a new strategy for the generation of more functional insulin-producing cells.

F-1074

LRIG1+ EMBRYONIC PROGENITORS AND POLYCOMB (PRC2) REPRESSION OF PANCREAS COMMITMENT

Sebastian Rieck[1], Anne E. Powell[2], Robert J. Coffey[2], Weipeng Mu[3], Andrew M. Fedoriw[3], Terry Magnuson[3], Christopher Wright[1]

[A]*Cell and Developmental Biology, Vanderbilt University Program in Developmental Biology,* [B]*Medicine & Cell and Developmental Biology, Epithelial Biology Center,* [1]*Vanderbilt University Medical Center, Nashville, TN, United States,* [2]*Genetics, Carolina Center for Genome Sciences, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*

β cell replacement therapies, specifically designed to decrease the incidence of diabetes-related disease, rely heavily on the precise understanding of transcriptional mechanisms critical for the normal construction and maintenance of healthy β cells. Chromatin-based transcriptional repression is a major control mechanism in both the 'regional allocation' of progenitors to specific organ fates and the increasingly resolved programs of 'intra-organ' cell differentiation. We have the unique ability to break down endodermal development *in vivo* into manageable units via new progenitor marker Lrig1 (a regulator of EGFR signaling). Our preliminary data strongly suggests that Polycomb Repressive Complex-2 (PRC2) is a central regulator of the type/strength of epigenetic repression of well-known central control genes of pancreas-differentiation in developing non-pancreatic tissue. We propose to investigate how PRC2 functions to repress pancreas programs, by cross-referencing various developing endodermal organs, with the goal to evoke plasticity *in vivo* or *in vitro* for reprogramming towards therapeutic cell populations, and inform the process of directed differentiation of ES cells. We aim to define the gene-inactive chromatin patterns on pancreatic transcription factor (TF) genes in various endodermal organs *in vivo*, focusing on early multipotent progenitor populations captured in a novel manner on the basis of the marker, Lrig1. The state of chromatin marking will provide key information on the potential to controllably reprogram various cell types, potentially even from other, quite distant, 'pre-organ territories' into pancreas and β-cells. We have unique access to newly generated flow-capture antibodies (Lrig1^{PE}), new well-behaved cell-selective Cre drivers (*Lrig1*^{CreERT2}), and floxed alleles for PRC2 components: *Ezh2*^{FLOX} and the novel *Eed*^{FLOX}. We will functionally interfere with PRC2-based patterning *in vivo*, to assess the epigenetic, gene expression, and associated phenotypic effects as multipotent progenitors fated to non-pancreatic organs begin to move to other cell states. *Lrig1*-driven *Ezh2* or *Eed* ablation will lead to identifying high-resolution methods to manipulate epigenetic coding associated with resistance to pancreas-commitment. More generally, our findings will be applicable to multiple different systems where transcriptional programs become differentiated during developmental and pathological transitions.

F-1075

USING SMALL MOLECULES TO INDUCE THE PROGRESSIVE DIFFERENTIATION OF HESCS INTO PANCREATIC β-CELL-LIKE PROGENITORS

Ian Chong[1], Cherie YL Lee[2], SYW Shiu[3], Kwok Ming Yao[1]
[A]*Biochemistry,* [B]*Obstetrics and Gynaecology,* [C]*Physiology,* [1]*University of Hong Kong, Hong Kong, Hong Kong*

As an alternative to transplantation from islet donors, the differentiation of ESCs to pancreatic β-cells can allow the latter to be infinitely supplied to diabetic patients without the restrictions of organ scarcity. 'Small molecules' are considerably more advantageous in differentiation strategies than protein factors, and we employed a variety of these inducers to bring the human ESC lines Val-3 and H9 into the definitive endoderm, as part of a progressive attempt to create β-like cells. Efforts to create this definitive endoderm have yielded results contradictory to previous reports. We show that between the TGF-β pathway activators IDE-1 and 2, the latter is more potent at inducing endoderm formation, though it does not surpass the capabilities of Stauprimide, a molecule originally thought to only serve a priming purpose in mouse ESCs. We have tried to explore the possible synergy between IDE-2 and Stauprimide, but interestingly this combination seems to hamper the expression levels of *Sox17* completely.
To aid in the discovery of small molecules and other biochemical inducers in promoting pancreatic differentiation, we are developing a cell-based platform that could assist in screening procedures. We have in hand a unique hESC line harboring a recombinase mediated cassette exchange system, which allows a gene of interest to be introduced into a genomic anchor site. By placing within this system a reporter construct for pancreatic gene expression; the promoters of *Pdx-1* and *Insulin 1* each driving a fluorescent protein, it will be possible to assess whether or not a molecule has potential in driving differentiation towards becoming a β-cell. Although the activation of *Pdx-1* is only

one of the checkpoints along the highway towards a β-like destination, it is imperative that we increase the *Pdx-1* expressing population since without doing so would reduce the efficiency of any further steps of differentiation. With the system that is being developed, it will greatly facilitate in drug discovery for aiding pancreatic regeneration.
We hypothesize that a molecule which could exert its effects through the protein kinase A (PKA) dependent pathway could indeed help coax several β-cell genes into expression. This conclusion was made after studies made on the secreted molecule sPdzd2 revealed that it could activate the expression of *Insulin 1, Glut2* and *MafA* in INS-1E cells, through a PKA guided mechanism also involving the critical pancreatic regulator *Pdx-1*. Given the inefficiency in which *Pdx-1*+ cells are being derived from the definitive endoderm as one attempts to differentiate along the pancreatic lineage, we would like to test molecules targeted towards the same PKA dependent mechanism that activates *Pdx-1*.

F-1076

ID3 DYNAMICALLY REGULATES STEM CELL FATE IN THE DEVELOPING FOREGUT

Quinn McNichols, Keith Gates, Challeng Yang, Duc Dong, Pamela Itkin-Ansari
Sanford-Burnham Medical Research Institute, La Jolla, CA, United States

During vertebrate development multipotent stem cells in the foregut must choose between pancreas and intestinal cell fate but how this is controlled is incompletely understood. We and others have previously shown that the basic Helix-Loop-Helix (bHLH) transcription factor PTF-1 promotes pancreas specification from multipotential foregut progenitors. To date, PTF1a is the only gene known to regulate this cell fate decision. Here we show that the bHLH antagonist Id3 acts in opposition to PTF1a to favor intestine over pancreas fate. We previously found a role for bHLH/Id3 interactions in the adult pancreas and pancreatic cancer and therefore considered the possibility that Id3 might also regulate pancreas development. In situ analysis for Id3 revealed that it is expressed a spacio-temporal manner consistent with a role for it in foregut development. To test the hypothesis that Id3 inhibits PTF1a activity *in vivo*, we created transgenic lines of *Danio rerio* (zebrafish) which inducibly express Id3 from the heatshock promoter. The transgenic animals also express GFP under control of the PTF1a promoter because the stability of GFP protein provides a lineage trace for pancreatic progenitors. We find that upon Id3 overexpression, a portion of pancreatic progenitors become misfated to intestine. The resulting phenotype precisely mimics that seen in zebrafish with diminished PTF1a activity or low PTF1a gene expression. To determine whether transient Id3 expression led to long-term changes in PTF1a function we measured expression of trypsin, a known PTF1a target. We find that trypsin gene expression is downregulated by Id3, consistent with antagonism of PTF1a protein by Id3. Collectively our results establish Id3 as a potent regulator of stem cell fate in the developing foregut.

Liver Cells

F-1081

IN VITRO EXPANSION OF SELF-RENEWING HEPATIC PROGENITOR CELLS BY CONTROLLING METABOLIC STATE

Hiroyuki Koike, Takanori Takebe, Yasuharu Ueno, Hideki Taniguchi
Yokohama City Univ, Yokohama, Japan

Induced pluripotent stem cells (iPSCs) are potential sources for production of mature hepatocytes. However, conventional iPSC-based approaches exceptionally costs too much including expensive cytokines to obtain the sufficient quantity and quality of iPSC-derived hepatocytes. Here, we took advantage of the unique metabolic properties of hepatic progenitor cells to develop an efficient approach for their in vitro expansion.
In order to reveal specific metabolic hallmarks in hepatic progenitor cells rather than mature hepatocyte, comparative metabolome and transcriptome analyses were performed. Among the various developmental stages, cells in the earliest fetal liver contained markedly lower amount of branched-chain amino acid (BCAA) than the mature hepatocytes. In turn, they expressed higher levels of branched chain aminotransferase (BCAT), a known energy

source during exercise in adult muscles. These unique metabolic profiles were also observed in iPSC-derived hepatic cells throughout hepatic differentiation. These results lead us to hypothesize that the supplementation of BCAA can purify and expand hepatic progenitor cells as they would specifically use them for an alternative energy source. To test our hypothesis, we exposed mouse fetal liver cells at early and later phase in liver development to BCAA-abundant condition with various concentrations. Surprisingly, supplementation of BCAA significantly promoted the expansion of self-renewing bipotent hepatic progenitor cells but not in the cells derived from the later phase of liver development. The effect of BCAA supplementation was also validated by the use of human iPSC-derived hepatic cells.
These preliminary results suggest that our approach provided simple and low-cost medium procedure that enabled us to expand hepatic progenitor cells, and finally large scale purification and production of hepatocytes aiming at future industrial or clinical application.

F-1082

HEPATOCYTE-DRIVEN REGENERATION IS SUFFICIENT FOR ADAPTATION TO CHRONIC INJURY

Branden D. Tarlow[1], Milton J. Finegold[2], Markus Grompe[1]
[1]Oregon Health & Sciences Univ, Portland, OR, United States, [2]Texas Children's Hospital, Houston, TX, United States

Chronic liver injury is associated with atypical ductal proliferations, or an oval cells response, in a variety of animal models and human diseases. While multiple transgenic mouse models and histologic studies suggest that persistent stem/progenitor activation is required for liver regeneration, direct evidence for progenitor necessity is lacking. Here we utilized the Fah-null mouse to generate chimeric livers with genetically distinct hepatocyte (Fah+/+) and ductal/progenitor (Fah-/-) compartments. We hypothesized that pharmacologic rescue of progenitor derived Fah-null hepatocytes would be required to maintain liver function.
Administration of the small molecule NTBC (nitisinone) allows for the survival of mature Fah-null hepatocytes and normalization of liver function; withdrawal of NTBC leads to cell-autonomous death of Fah-null hepatocytes and the selective proliferation of Fah+/wildtype hepatocytes (when present).
Results: To generate chimeras, Fah-/- mice received congenic Fah+/+ Rosa-lacZ or Rosa-mTomato donor hepatocytes by intrasplenic injection. After 10-12 weeks of repopulation, we observed 95-99% replacement with donor hepatocytes, with residual host hepatocytes found in focal areas fed by distal vasculature. mTomato+ donor cells were restricted to HNF4a+ cells with hepatocytic morphology, demonstrating the hepatocytes were specifically replaced. No transplanted donor cells were found to express markers found on progenitors (CK19/Osteopontin), endothelium (CD31), or stellate cells (GFAP) in multiple animals surveyed. Then, to induce prototypical oval cell activation, chimeric animals were treated twice-weekly for 10 weeks with carbon-tetrachloride (CCL4) for 4 weeks or with the choline-deficient, ethionine supplemented (CDE) diet. Surprisingly, no difference in weight or survival was observed in injured mice that received NTBC compared to those that received injury alone, demonstrating that rescue of progenitor derived hepatocytes does not alter measures of global liver function. In CCL4-treated animals, small clusters comprising <1% of the hepatocyte mass were identified as host/progenitor derived, regardless of NTBC rescue. In CDE treated animals, we failed to find clear histologic evidence of progenitor-to-hepatocyte differentiation. BrdU-pulse at the end of injury showed active proliferation of both ductal cells and hepatocytes.
In conclusion, our study suggest progenitor-to-hepatocyte differentiation is a relatively rare event that is unlikely to occur at sufficient rates to be physiologically important in these commonly studied mouse models of oval cell activation.

F-1084

OCT4, SOX2, KLF4 AND C-MYC IMPROVE DIRECT CONVERSION OF MOUSE EMBRYONIC FIBROBLASTS TO HEPATOCYTES (IHEP) WHEN CO-EXPRESSED WITH HEPATIC-SPECIFIC TRANSCRIPTION FACTORS

FELIPE SERRANO, Marina Blazquez, Jose Castell, Roque Bort
IIS La Fe, Valencia, Spain

Direct conversion of mouse fibroblasts to iHep by expression of hepatic-specific transcription factors is limited by senescence and their limited proliferation. Here, we show that retroviral coexpression with Oct4, Sox2, Klf4 and c-Myc bypass senescence and maintain high proliferation rates in iHep. Induced cells display hepatocyte morphology, express endogenous hepatic transcription factors as well as Albumin, Tat, Slc10a1, Cyp7a1 or Ugt1a1. Furthermore, these cells acquire competence for xenobiotic metabolism and other hepatic functions. In parallel iHep lose the expression of fibroblast markers and do not express endogenous pluripotent genes. The expression of Oct4, Sox2, Klf4 and c-Myc with hepatic-specific transcription factors not only limits senescence but also improves the phenotype of iHep. Taken together, we show that it is possible to bypass iPSC formation redirecting reprogramming towards the hepatic lineage by coexpression of Hhex, Gata4, Foxa2, HNF4α, HNF1α and HNF6.

F-1085

GENERATION OF FUNCTIONAL HEPATOCYTE-LIKE CELLS FROM HUMAN PLURIPOTENT STEM CELLS IN A SCALABLE CULTURE

Massoud Vosough, Behshad Pournasr, Nasser Aghdami, Hossein Baharvand
Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institut, Royan Institute, Tehran, Iran, Islamic Republic of

BACKGROUND & AIMS: Recent advances in human embryonic and induced pluripotent stem cell-based therapies in animal models of hepatic failure have led to an increased appreciation of the need to translate the proof-of-principle concepts into more practical and feasible protocols for scale up and manufacturing of functional hepatocytes. In this study, we describe a scalable production of functional hepatocyte-like cells from the human pluripotent stem cells in a stirred bioreactor.
METHODS: To promote the initial differentiation of human pluripotent stem cells in a carrier-free suspension stirred bioreactor into definitive endoderm, we used rapamycin for “priming” phase and activin A for induction. The cells were further differentiated into hepatocyte-like cells in the same system. Hepatocyte-like cells were characterized and then purified based on their physiological function, the uptake of DiI-acetylated low-density lipoprotein by flow cytometry without genetic manipulation or antibody labeling. The sorted cells were transplanted into the spleens of mice with acute liver injury from carbon tetrachloride.
RESULTS: The differentiated hepatocyte-like cells had multiple features of primary hepatocytes e.g., the expression patterns of liver-specific marker genes, albumin secretion, urea production, collagen synthesis, indocyanin green and low-density lipoprotein uptake, glycogen storage, and inducible cytochrome P450 activity. They increased the survival rate, engrafted successfully into the liver, and continued to present hepatic function (i.e., albumin secretion after implantation).
CONCLUSIONS: This amenable scaling up and outlined enrichment strategy provides a new platform for generating functional hepatocyte-like cells. This integrated approach may facilitate biomedical applications of the human pluripotent stem cell-derived hepatocytes.

F-1086

CHOLESTEROL-SECRETING AND STATIN-RESPONSIVE HEPATOCYTES FROM HUMAN ES AND IPS CELLS TO MODEL HEPATIC INVOLVEMENT IN CARDIOVASCULAR HEALTH

Winfried H. Krueger, Borko Tanasijevic, Vanessa Barber, Anthony Flamier, Xinsheng Gu, Jose Manautou, Theodore Rasmussen
Department of Pharmaceutical Sciences, Univ of Connecticut, Storrs, CT, United States

Hepatocytes play a central and crucial role in cholesterol and lipid homeostasis and their proper function is of key importance for cardiovascular health. In particular, hepatocytes (especially periportal hepatocytes) endogenously synthesize large amounts of cholesterol and secrete it into circulating blood via apolipoprotein particles. Cholesterol-secreting hepatocytes are also the clinically-relevant cells targeted by statin treatment *in vivo*. The study of cholesterol homeostasis is largely restricted to the use of animal models and immortalized cell lines that

do not recapitulate those key aspects of normal human hepatocyte function that result from genetic variation of individuals within a population. Hepatocyte-like cells (HLCs) derived from human embryonic and induced pluripotent stem cells can provide a cell culture model for the study of cholesterol homeostasis, dyslipidemias, the action of statins and other pharmaceuticals important for cardiovascular health. We have analyzed expression of core components for cholesterol homeostasis in untreated human iPS cells and in response to pravastatin. Here we show the production of differentiated cells resembling periportal hepatocytes from human pluripotent stem cells. These cells express a broad range of apolipoproteins required for secretion and elimination of serum cholesterol, actively secrete cholesterol into the medium, and respond functionally to statin treatment by reduced cholesterol secretion. Our research shows that HLCs derived from human pluripotent cells provide a robust cell culture system for the investigation of the hepatic contribution to human cholesterol homeostasis at both cellular and molecular levels. Importantly, it permits for the first time to also functionally assess the impact of genetic polymorphisms on cholesterol homeostasis. Finally, the system will also be useful for mechanistic studies of heritable dyslipidemias, drug discovery, and investigation of modes of action of cholesterol-modulatory drugs.

F-1087

THE BILIARY COMPARTMENT IS THE ORIGIN OF LIVER PROGENITOR CELLS AND POTENTIALLY CONTRIBUTES TO NEWLY GENERATED HEPATOCYTES IN HEPATIC REGENERATION

Daniel Rodrigo-Torres, Silvia Affò, Carlos Enrich, Pere Ginès, Jorge Ferrer, Ramon Bataller, **Pau Sancho-Bru**
IDIBAPS, Barcelona, Spain

Background and aims: Liver progenitor cells (LPC) proliferate in response to liver injury when the regenerative capacity of hepatocytes is impaired. However, whether they derive from the biliary compartment and how they contribute to liver parenchyma homeostasis and regeneration is still controversial. By tracing Hepatocyte Nuclear Factor (Hnf)1β biliary cells we aim at assessing the contribution of biliary compartment cells to the generation of LPC and hepatocytes during liver homeostasis, liver injury and regeneration.
Methods: HNF1β expression was evaluated in human livers. The fate of Hnf1β biliary cells was assessed in Hnf1βCreER/Rosa26R mice. Mice were treated with tamoxifen to induce persistent expression of the reporter gene β-galactosidase/Yfp in Hnf1β cells. LPC contribution was evaluated in different liver injury models: acute acetaminophen (500mg/kg) and CCl4 (0.5ml/kg); DDC (4 weeks) and CDE (3 weeks) diets, chronic CCl4 (8 weeks) and partial hepatectomy (PH). YFP+ cells were sorted from DDC-treated mice for further characterization.
Results: Ductular reaction cells in human cirrhotic livers for alcohol intake co-expressed HNF1β and Ephitelial cell adhesion molecule (EpCAM). However, HNF1β expression was not present in newly generated EpCAM-positive hepatocytes. To assess the specificity of our animal model, Cre immunohistochemistry was assessed showing that Cre expression was restricted to biliary epithelial cells and not re-expressed after tamoxifen induction. Tamoxifen-induced Hnf1βCreERYFP marking efficiency was 29±10% as assessed by dual cytokeratin 19 (CK19)/YFP staining. Under healthy liver homeostasis conditions, no Hnf1β-derived hepatocytes were observed. After liver injury, ductular reaction cells were positive for YFP or β-galactosidase (β-gal) and co-expressed LPC markers (A6, TROP2) and biliary markers (Sox9, CK19) indicating that ductular reaction cells were derived from Hnf1β-positive cells. In mouse models of acute liver injury, partial hepatectomy and DDC diet, Hnf1β cells gave rise to LPC but not to newly generated hepatocytes. However, CDE diet treated animals showed presence of positive YFP positive LPC as well as a small population of Hnf1β-derived hepatocytes in the periportal area as assessed by Hepatocyte Nuclear Factor (Hnf)4α and YFP staining. Gene expression analysis showed that DDC and CDE diets, as well as PH had an induction of LPC markers. Moreover, FACS-sorted purified YFP-positive cells from DDC-treated mice expressed LPC markers (CD133, EpCAM, TROP2) and hepatobiliary markers (HNF1β, CK19, SOX9).
Conclusion: Hnf1β biliary cells do not participate in liver parenchymal homeostasis in healthy liver but give rise to ductular reaction cells with LPC characteristics after liver injury. However, under these experimental conditions the biliary compartment contributes minimally to the regeneration of the liver parenchyma and only under specific liver injury stimuli, Hnf1β cells can generate periportal hepatocytes, yet, at a low frequency. These results support the hypothesis that although LPC participate in liver regeneration and can generate mature hepatocytes, pre-existing hepatocytes are the main source of regenerating cells after liver injury.

F-1088

MODELING ALPHA-1 ANTITRYPSIN DEFICIENCY WITH HUMAN INDUCED PLURIPOTENT STEM CELLS

Tamara N. Taketani
Pediatrics, UCSD, La Jolla, CA, United States

As background, a major obstacle to the development of new therapies is the poor understanding of how genetic modifiers alter the outcome of various diseases. A classic example is AAT deficiency, a monogenic metabolic liver disease in which the mutant gene and its product are known, but where clinical progression and outcome are extremely variable and thought to be influenced by genetic modifiers. There is no treatment for AAT deficiency, and liver transplantation is currently the only available therapeutic option for liver failure caused by AAT deficiency.
Despite being the leading genetic cause of liver disease in children, AAT deficiency occurs infrequently when compared to sporadic liver diseases. The relatively low incidence of AAT deficiency makes it impossible to obtain insight into the genetic factors that may affect progression of disease from genome-wide association studies (GWAS).
However, study of AAT deficient human induced pluripotent stem cell (hIPSC) derived hepatocytes may overcome this limitation by identifying cellular phenotypes that correlate with clinical severity of disease in existing AAT deficient patients. This approach can help unravel genetically determined susceptibility factors that affect disease progression, and test whether targeting these pathways pharmacologically can revert liver disease in AAT deficiency and potentially in other liver diseases.
For this purpose, we have generated hIPSC lines from existing AAT patients with variable degrees of liver disease, including those without evidence of liver damage and those who have suffered a more aggressive course leading to significant liver disease. We will use control and AAT hIPSC-derived hepatocytes to probe the hypothesis that the significant heterogeneity seen in disease progression due to AAT mutations is related to genetically determined variability of fundamental biological hepatocyte processes involved in cellular disposal, stress response, and cell survival pathways.
Our studies can potentially impact the way we approach AAT deficiency by developing predictive diagnostics through discovery of biomarkers that can identify AAT deficient patients at risk for severe liver disease, and by promoting therapeutic candidate discovery through validation of new and existing therapeutic targets for AAT deficiency in live human hepatocytes.

F-1091

HIGHLY EFFICIENT DIFFERENTIATION OF HUMAN PSC INTO HEPATOCYTE-LIKE CELLS BY SELECTION OF CXCR4+ DEFINITIVE ENDODERM CELLS

Annett Kurtz, Andreas Bosio, **Sebastian M. Knoebel**
Miltenyi Biotec GmbH, Bergisch-Gladbach, Germany

Hepatoytes fullfill central metabolic functions of the body such as synthesis of proteins, fatty acids and bile acids, as well as detoxification through class I and II transformation reactions. The loss of active hepatocytes during inherited or acute liver disease or intoxication leads to severe pathopysiological effects. Therefore, the etiology of liver disease is of major interest. Lack of healthy primary hepatocytes neccessitates alternative cell sources for in vitro studies. Hepatocyte-like cells (HLC) derived from in vitro differentiated pluripotent stem cells (PSC) constitute a potential source for disease in a dish modelling, drug screening and future cell replacement strategies. Generation of HLC in vitro is usually achieved by exposing PSC to high doses of ActivinA in combination with FGF and Wnt pathway activation. This leads to induction of nascent defintive endoderm (DE) that can be subsequently specified to hepatic endoderm (HE), hepatoblasts and finally metabolically active HLC. However, varying efficiencies of PSC differentiation paradigms are well documented and caused e.g. by cell line predisposition, lot-to-lot variances of differentiation media components and experimental inconsistencies. Using a DE induction medium containing ActivinA/FGF/CHIR99021 (GSK3-beta inhibitor) we were able to derive DE cells at varying frequencies, i.e. between 30 to 60% at day 5 of differentiation. These cells co-expressed FoxA2, Sox17 as detected by immunofluorescence and quantified by flow cytometry analyses. We found the cell surface marker CXCR4 (CD184) to be expressed on

all FoxA2+/Sox17+ cells which constituted on avergae 80% of the total CXCR4+ population at this stage. In order to compensate for the inherent variability of DE induction we established protocols for immunomagnetic isolation of CXCR4+ cells to purities between 95-98 %. Thereby, we could demonstrate that the hepatogenic potential is limited to the CXCR4+ cell population, and that isolation of these nascent progenitor cells allows for reproducible generation of highly enriched HLC cultures.

F-1092

DIFFERENTIATION OF PLURIPOTENT STEM CELLS AND HEPATIC PROGENITOR CELLS HEPARG INTO CHOLANGIOCYTES.

Noushin DIANAT[1], Hélène DUBOIS-POT-SCHNEIDER[2], Aurélien RAVEUX[1], Anne DUBART-KUPPERSCHMITT[1], Anne CORLU[2], Anne WEBER[1]

[1]Hôpital Paul Brousse, INSERM U972, Villejuif, France, [2]Hôpital Pontchaillou, INSERM U991, Rennes, France

During the liver development, hepatocytes and cholangiocytes, the two types of hepatic epithelial cells, derive from bipotent hepatic progenitors (hepatoblasts). Although cholangiocytes represent a small fraction of the total cellular component of the liver, these cells actively regulate bile composition through a process of absorption and secretion. Cholangiocytes can also reabsorb bile acids, a process that is important in cholestatic liver diseases. Therefore, understanding the molecular mechanisms regulating the differentiation of hepatic progenitors into functional cholangiocytes would be a great help not only to understand the mechanisms underlying the pathologies, but also to find novel treatments for patients with biliary diseases.
In this study, our goal was to define conditions to differentiate hepatic progenitor cells into cholangiocytes. To this aim, we used human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC)-derived hepatoblasts, as well as HepaRG cells (a cell line with adult liver cell phenotype capable of being reverted into hepatoblasts).
Hepatoblasts were first generated from hESCs and hiPSCs in defined conditions already established in the laboratory and characterized for specific markers of bipotency such as α-fetoprotein (AFP), HNF4α, HNF6 and CK19. After passage on day 12 of differentiation, hepatoblasts were then plated on a collagen I matrix and different protocols were tested to induce cholangiocyte differentiation.
In the presence of various cytokines, hepatic progenitors proliferated and after a few days co-expressed HNF6, a transcription factor expressed in hepatoblasts and cholangiocytes, CK19 also expressed in both cell types, and CK7, specific to cholangiocytes. Expression of these markers was correlated with the extinction of AFP and of HNF4α, markers normally expressed during the differentiation of hepatoblasts into fetal hepatocytes. Differentiating cholangiocytes also expressed Notch2, EpCAM, SOX9 and CFTR.
Conditions to induce differentiation of HepaRG progenitor cells toward cholangiocytic lineage were also defined. In the presence of cytokines and sodium butyrate, expression of Sox9, Notch2, CFTR and GGT1 were increased whereas hepatic markers like Albumin and Aldolase B were strongly repressed.
Functionality tests were carried out in a three dimensional matrix with cholangiocytes derived from hESCs and HepaRG cells. Both cell types were capable of forming cysts and tubules after 20 days of culture. Polarization of cholangiocytes was visualized by transport of rhodamine 123 in the lumen of the cysts.
This study paves the way for the generation of cholangiocyte cell lines derived from hiPS cells from patients with genetic cholestatic defects as disease models.

F-1093

HIPPO PATHWAY INVOLVEMENT IN LIVER DEVELOPMENT AND TUMORIGENESIS IN ZEBRAFISH

Andrew G. Cox[1], Sebastian Beltz[1], Katie L. Hwang[1], Kimberley Evason[2], Didier Stainier[2], Trista E. North[3], Wolfram Goessling[1]

[1]Genetics Division, Brigham and Womens Hospital, Harvard Medical School, Boston, MA, United States, [2]Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA, United States, [3]Department of Pathology, Beth Israel Deaconess Medical Center; Harvard Medical School, Boston, MA, United States

The Hippo pathway is a novel signaling cascade that regulates cell proliferation, organ size and tumorigenesis in mammals. In mice, deregulation of the Hippo pathway in the liver has been shown to potently induce tumor formation. However, the molecular mechanism by which the Hippo pathway regulates hepatic growth is not well understood. In this study, we have examined liver development in NF2-/- mutant zebrafish, which exhibit systemic activation of Yap, and found that liver size was increased by 5 days post fertilization (dpf). Histological evaluation of the NF2-/- mutant zebrafish embryos revealed that the enlarged liver was composed of an extended biliary network. In order to examine the cell-autonomous effects of Yap activation in hepatocytes, we generated Tg(fabp10a:YapS87A) fish and found that liver size increased dramatically by 5 dpf. In contrast to the NF2-/- mutant, histological and FACS analysis revealed that the enlarged liver in the Tg(fabp10a:YapS87A) embryos was composed of a greater number of hepatocytes. Chemical exposure to erlotinib (10 µM), which inhibits EGFR, rescued the hepatomegaly phenotype in 5 dpf Tg(fabp10a:YapS87A) embryos. To gain a greater insight into how these developmental studies relate to tumorigenesis, we compared NF2+/- mutants and Tg(fabp10a:YapS87A) adults and found that both lines were predisposed to liver tumor formation (both spontaneous and DMBA-induced). Consistent with mammalian studies, the liver tumors that formed were predominantly intrahepatic cholangiocarcinomas. Together, these studies reveal an evolutionarily conserved role of the Hippo pathway at the interface between liver development and tumorigenesis in zebrafish.

F-1094

A ROSE IS A ROSE, OR IS IT? HOW TO JUDGE SUCCESS IN STEM CELL DIFFERENTIATION

Samrina Rehman[1], Justyna Jozefczuk[2], Wasco Wruck[3], James Adjaye[4], Hans V. Westerhoff[1]
[1]University of Manchester, Manchester, United Kingdom, [2]Max Planck Institute Molecular Genetics, Berlin, Germany, [3]Charité Universitätsmedizin, Berlin, Germany, [4]Heinrich Heine University, Düsseldorf, Germany

In natural differentiation stem cells acquire the properties that are characteristic of tissues, and enable tissue function. In stem cell differentiation in the laboratory much the same is attempted by cultivating of cells under conditions similar to those in natural differentiation, but may not be quite the same. Thereby it becomes an issue whether the cells resulting from the latter type of differentiation are fully identical to the cells that naturally occur in the target tissue.
This study identifies and tries out several different criteria to judge whether they are. These range from alterations in the expression level of mRNAs that are held to be characteristic of the tissue, to estimates of the functional performance of the cells. The issue is confounded by the fact that cells of most tissue types are themselves heterogeneous, in terms of gene expression if not also in terms of function.
We show that cells according to the usual criteria are fully differentiated into the tissue one aimed for, but are far from the desired state according to other criteria. We propose that for perfect in vitro differentiation all criteria should be satisfied, or the ones most closely related to function.

F-1095

MODELING OF MONOGENIC PRIMARY HEPATIC STEATOSIS WITH hIPSC DERIVED HEPATOCYTE LIKE CELLS

Sanna C. Toivonen[1], Maarit Hölttä-Vuori[2], Marita Lipsanen-Nyman[3], Elina Ikonen[4], Timo Otonkoski[5]
[A]Biomedicum Stem Cell Center, [B]Institute of Biomedicine/Anatomy, [C]Children's Hospital, [D]Institute of Biomedicine, Anatomy, [E]Biomedicum Stem Cell Center, Children's Hospital, [1]University of Helsinki, Helsinki, Finland

Background: Hepatocyte like cells (HLCs) derived from human pluripotent stem cells (hPSCs) provide tools for drug screening, toxicology assays and cell transplantation therapies. In addition, hiPSCs derived from patients with hep-

atic diseases enable studies of disease mechanisms. We aimed to develop an iPSC based model to study primary hepatic steatosis. For this purpose, we used hiPSCs derived from fibroblasts of patients with Mulibrey nanism (MUL), an autosomal recessive growth disorder enriched in the Finnish population, caused by mutations in the TRIM37 gene. One of the hallmarks of the disease is the development of fatty liver at young age, associated with insulin resistance and type 2 diabetes.
Methods: Five hiPSC lines were derived from two MUL patients carrying the homozygous TRIM37 mutation c.493-2A>G. Hepatic differentiation was induced by a stepwise protocol. The cells were first differentiated into definitive endoderm and further into hepatic progenitors by 5 days with BMP4 and FGF2 and then further 5 days with HGF. Final maturation was induced by HGF and Oncostatin M. Markers of hepatocytic differentiation and lipid synthesis were analyzed in at least three independent experiments.
Results: Differentiated MUL-HLCs cells expressed genes characteristic for fetal hepatocytes, such as Albumin, AFP and HNf4a and secreted albumin similarly as healthy control HLCs. Hepatocytic differentiation was associated with an increasing level of ApoB, as sign of initiation of lipoprotein synthesis, and the expression of the lipid droplet surface protein ADRP. MUL-HLCs were larger than the control cells and contained a high amount of lipid droplets. Lipid analysis revealed elevated amount of triglycerides in MUL-HLCs compared to CNTR-HLCs.
Conclusions: iPS cells derived from MUL patients provide a platform for studies of mechanisms leading to hepatic steatosis, a primary event in the development of insulin resistance and the metabolic syndrome.

Intestinal/Gut Cells

F-1101

A DEDICATED ROLE FOR TRANSCRIPTION FACTOR CDX2 IN INTESTINAL STEM CELL FUNCTIONS

Adrianna K. San Roman[1], Ramesh A. Shivdasani[2]
[1]*Developmental and Regenerative Biology Program and Biological and Biomedical Sciences Program, Harvard University, Boston, MA, United States,* [2]*Department of Medical Oncology and Center for Functional Cancer Epigenomics, Dana Farber Cancer Institute and Harvard Medical School, Boston, MA, United States*

Intestinal crypt stem cells (ISCs) replenish the adult gut epithelium every few days, express LGR5, rely on Wnt signaling to replicate frequently, and represent a valuable model for tissue differentiation and stem-cell properties. Differentiation of ISCs and their immediate progeny into mature, post-mitotic cells requires precise control of gene expression at each successive stage. One transcription factor (TF) essential for gene activation in mature enterocytes, CDX2 (caudal type homeobox 2), controls many intestinal genes and maintains active chromatin marks at their enhancers. CDX2's role in ISCs logically anticipates its enterocyte functions, but it is unclear how ISCs prepare chromatin for TF binding and activity in their mature descendants. We find that *Cdx2*$^{-/-}$ ISCs are markedly impaired in the ability to replicate or produce mature cell types. This defect contrasts with CDX2 requirements in activating genes that mediate digestion and nutrient absorption in mature enterocytes; thus, CDX2 fulfills distinct roles in ISCs and their differentiated progeny. In agreement with this result, chromatin immunoprecipitation coupled with massively parallel sequencing (ChIP-seq) revealed that over 80% of CDX2 binding sites in isolated LGR5+ mouse ISCs differ from those in mature intestinal villus cells. CDX2 binding in ISCs *in vivo* localized near well-characterized intestinal marker genes *Lrig1 and HopX*, implying a direct role in regulating their expression. Other transcriptional targets suggest that CDX2 modulates ISC self-renewal through the epidermal growth factor (EGF) signaling pathway. Together, these observations demonstrate the versatility of a master TF such as CDX2 in distinct aspects of the functions of ISCs and their derivative cells.

F-1102

GENERATION OF INTESTINAL TISSUE FROM INDUCED PLURIPOTENT STEM CELLS

Robert Barrett, Nicole Yeager, Loren Ornelas, Dhruv Sareen, Clive Svendsen, Stephan Targan
Cedars Sinai Med Ctr, Los Angeles, CA, United States

Introduction: Crohn's Disease (CD) is a chronic inflammatory disorder that can affect any part of the gastrointestinal tract. Despite extensive research, the causes of CD remain elusive. One relatively unexplored area of study is what role the intestinal epithelium may play in this disease. The aim of this study is to generate intestinal epithelium from control individuals with a view to eventually comparing it to epithelium generated from genetically defined IBD patients.
Method: Induced pluripotent stem cells (iPSCs) were generated from control individuals using an episomal plasmid-based reprogramming system. The generation of intestinal tissue from iPSCs is a multi-step process which involves directing iPSCs into definitive endoderm, then hindgut tissue and subsequently into three dimensional intestinal "organoids." To generate definitive endoderm, control iPSC lines were incubated with 100ng/ml of Activin A and 25ng/ml of Wnt3A under low serum conditions for three days. To generate hindgut tissue, definitive endoderm was incubated in Advanced DMEM/F12 media with 10% FCS with or without 500ng/ml of Wnt3A and FGF4 for a further three to four days. During the hindgut differentiation protocol, epithelial "tubes" became visible after three to four days. These epithelial tubes were harvested and then cultured in a three dimensional Matrigel matrix containing the intestinal trophic factors noggin, RSpondin-1, EGF and B27.
Results: The generation of definitive endodermal tissue in all lines was confirmed by the upregulation of Sox17, FoxA2 and Goosecoid mRNA in these cells. Furthermore, it was shown that the vast majority of these cells co-expressed FoxA2 and Sox17 by immunocytochemistry and CXCR4 and Sox17 by flow cytometry. The generation of hindgut tissue was demonstrated by both the presence and upregulation of the hindgut intestinal marker CDX2. The epithelial tubes that were cultured in a three dimensional Matrigel matrix steadily increased in size over time to generate spherical intestinal organoids (approx. 500μm). The four differentiated intestinal cell subtypes were found in these organoids. Goblet cells (Muc2+), Paneth cells (Lysozyme+), enterocytes (FABP2+) and enteroendocrine cells (Chromogranin A+) were all observed in these intestinal organoids at various different timepoints. Furthermore, the presence of apical gap junctions as illustrated by ZO-1 and Occludin was observed in these organoids also.
Conclusion: Intestinal organoids can be generated from control individuals. This tissue contains enterocytes with polarized gap junctions and the three additional cell types. This will be used to develop functional assays to assess each component of the organoid system thereby providing a system to study the different cell components of CD patients.

F-1103

CONTROL THE BEHAVIOR OF INTESTINAL STEM CELLS

Xiaolei Yin[1], Henner F. Farin[2], Hans Clevers[2], Robert Langer[1], Jeffrey M. Karp[3]
[1]Koch Institute, Massachusetts Institute of Technology, Cambridge, MA, United States, [2]Hubrecht Institute for Developmental Biology and Stem Cell Research and University Medical Centre Utrecht, Utrecht, Netherlands, [3]Center for Regenerative Therapeutics and Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA, United States

The intestinal epithelium is a single layer of epithelial cells composed of both absorptive and secretory cell types, which are fueled by intestinal stem cells residing at the base of crypts. Recently, Lgr5+ stem cells were found to maintain the homeostasis of intestinal epithelium. Isolated intestinal crypts or single Lgr5+ intestinal stem cells can be cultured in vitro in the presence of EGF, Noggin and R-spondin1, and form organoids with crypt-villus structures containing all the cell types in the intestinal epithelium. While cells cultured in this condition can be readily expanded for multiple passages in the form of organoids, existing culture conditions provide little to no control over the self-renewal and differentiation of the cells. Typical cultures consist of very heterogeneous cell populations including both stem cells and differentiated cells. In addition, the maintenance of stem cells depends on neighboring Paneth cells, which restricts the ability to efficiently expand intestinal stem cells. Indeed, the culture of single intestinal stem cells was highly inefficient in the absence of Paneth cells (with a reported colony-forming efficiency of 1-6%). We speculate that conventional culture system provides essential, but not adequate cues to sustain the self-renewal of Lgr5+ stem cells, and other factors are needed to maintain the self-renewal status of intestinal stem

cells. To identify such factors, we tested selected small molecules that target relevant signaling pathways. We identified a combination of small molecules which greatly promoted the proliferation and self-renewal, as well as the culture efficiency of intestinal stem cells in vitro. Specifically, when isolated Lgr5-GFP crypts were cultured in the presence of the small molecules, cell proliferation as well as GFP expression of the cells significantly increased, with nearly pure GFP+ cells (>97%) in the culture which showed an over 30 fold increase of the number of Lgr5+ stem cells in the culture. The colony-forming efficiency from single stem cells was significantly increased (>100 fold), and greater than 90% of the live cells formed GFP+ colonies. This appears to be the most efficient colony formation that has been reported for Lgr5+ stem cells. In this culture condition, Lgr5-GFP+ stem cells were present throughout the entire colony with minimal presence of Paneth cells, and without the presence of other differentiated cell types, suggesting homogenous Lgr5+ stem cells in the culture. When transferred to the conventional culture condition which allows spontaneous differentiation of stem cells, the expanded stem cells differentiated into all cell types in the intestinal epithelium, characterized by the expression of differentiation markers. Furthermore, we successfully identified several differentiation conditions by using a combination of small molecules. Intestinal stem cells cultured in these conditions specifically differentiated into enterocytes, goblet, enteroendocrine and Paneth cells. These results indicate that stem cells cultured with small molecules are fully functional and preserve the ability to generate all the cell types in the intestinal epithelium. We are now studying the mechanisms of these small molecules and applying this culture system to stem cells from other tissues. Taken together, we have established a new method to homogenously expand stem cells and to control their fate by harnessing small molecules that target specific signal transduction pathways.

F-1104

MESENCHYMAL STROMAL CELLS THERAPY IMPROVES COLONIC EPITHELIAL REGENERATION AND SUPRESSES RADIATION-ACTIVATED T CELLS: NEW INSIGHTS FOR PELVIC RADIATION DISEASE TREATMENT

DEMARQUAY Christelle, Alexandra SEMONT, Christelle DURAND, Raphaëlle BESSOUT, Marc BENDERITTER, **Noelle MATHIEU**
IRSN, Fontenay aux roses, France

Background: The efficacy of radiotherapy requires an optimal compromise between tumor control and normal tissue injury. Non-neoplastic tissues around an abdomino-pelvic tumor can be damaged by ionizing radiation, leading to chronic gastrointestinal complications which affect quality of life with substantial mortality. Chronic radiation proctitis is an increasing problem as more patients receive radiotherapy and survive longer after treatment. There is no unified approach to the assessment and treatment of this disease, characterized mainly by severe tissue damages and uncontrolled inflammation. Stem cell-based approaches using mesenchymal stromal cells (MSC) from bone marrow are promising cell therapy tools.
Objectives: In this study, we tested the therapeutic benefit of mesenchymal stromal cells (MSC) treatment and proposed molecular mechanisms of action.
Methods: MSC efficacy was tested in an experimental model of radiation-induced similar severe colonic ulceration to those observed in patients. Rats were subjected to 27Gy colorectal irradiation. In this model, MSC ($5x10^6$ cells) were administered intravenously, immediately or three weeks (established lesions) after irradiation and analysis were performed 1, 2, 8 or 21 weeks after irradiation.
Results: MSC therapy reduces irreversible radiation-induced colonic ulcers and prolongs animal survival. MSC engraft in lung and colonic mucosa but also mobilize endogenous MSC that could endure the benefit over time. MSC benefit might be rapidly induced after MSC infusion by the secretion of a broad range of molecules that can act through abscopal and local effects. We also observed stimulation of secretion of epithelial growth factors as well as steroid anti-inflammatory molecules by colonic mucosal cells after MSC treatment. Secretion of these molecules is associated to stimulation of proliferation of colonic epithelial cells positive for SOX9 progenitor/stem cell (ISC) marker and decrease of T lymphocytes infiltrate. Altogether our results demonstrated that MSC benefit improves the regenerative process by healthy margins rather than a decrease of fibro-necrosis to reduce severe irradiation gut damages.

Conclusions: MSC treatment stimulates endogenous host progenitor cells (MSC and ISC) and reduces inflammation (T cells) to improve the regenerative process after severe irradiation gut damages. Results of this study constitute a first approach to arguing in favor of the use of MSC for compassionate applications to limit/reduce colorectal damages induced by pelvic radiotherapy.

F-1105

RESERVE INTESTINAL STEM CELLS IDENTIFIED BY SURFACE MARKER IN INJURY MODEL

Fengchao Wang[1], Xi C. He[1], Ryohichi Sugimura[1], John M. Perry[1], Andrew Box[1], Craig Semerad[1], Jeffrey S. Haug[1], Jian Yu[2], Linheng Li[1]

[1]Stowers institute for medical research, Kansas City, MO, United States, [2]Department of Pathology, University of Pittsburgh Medical Center,, Pittsburgh,PA, PA, United States

Reserve stem cells in adult tissue usually play a marginal role in daily tissue maintenance but a major role in regeneration in response to tissue damage. Multiple markers including Bmi-1, mTert, Hopx, Lrig1 have been reported to label a reserve intestinal stem cells (ISC) population located above Paneth cells. However, these markers have also been expressed in active Lgr5^{hi} ISCs intermingled with Paneth cells. Reliable surface markers to enrich ISCs that specifically play a reserve role are still lacking. Herein, we aim to characterize the non-Lgr5^{hi} ISC population, including reserve ISCs, by surface markers and functional analysis. With our improved culture method, we found not only that Lgr5-GFP^{hi} cells enrich cycling ISCs, but also that Lgr5GFP$^{med/lo}$ cells in Lgr5GFP^{+} crypt form enteroids that can be maintained in long-term culture. Lgr5GFP$^{med/lo}$ cells had more heterogeneities in surface marker staining, including CD44,CD24,CD166, and GRP78, whereas clonogenic Lgr5GFP$^{med/lo}$ cells were enriched in CD44^{+}CD24^{lo}CD166^{+}GRP78^{-} population. Their low level Lgr5 expression was also confirmed by gene profiling test. High doses of radiation (11Gy) eliminated the majority of Lgr5GFP^{hi} cells in Paneth cell zone. However, the subsequent cellular proliferation following irradiation initiated mainly at the position above Paneth cells, indicating that the major population that survived insult and maintained the clonogenic capacity was at +4 ISC position. The CD44^{hi}CD24^{lo}CD166^{+}GRP78^{-} population isolated at day 3-4 post irradiation expressed low levels of Lgr5 as well as differentiation markers including DLL1, and was able to generate enteroid. In conclusion, we found that CD44^{+}CD24^{lo}CD166^{+}GRP78^{-} cells enriched reserve ISCs under injury condition.

History of Stem Cell Research

F-1106

ACTIVATION OF THE WNT/β-CATENIN-SIGNALING PATHWAY TRIGGERS EXPRESSION OF HUMAN INTESTINAL EPITHELIAL STEM CELL MARKERS.

Amel GUEZGUEZ[1], Frederic Paré[1], Yannick Benoit[2], Blanche Sénicourt[1], Nuria Basora[1], Jean-Francois Beaulieu[1]

[1]Anatomy and Cell Biology, Université de Sherbrooke, Sherbrooke, QC, Canada, [2]Stem cell and Cancer research institut, McMaster University, Hamilton, ON, Canada

The small intestine consists of two histological
compartments composed of the crypts and the villi. Crypts are invaginations
into the submucosa while villi are finger-like protrusions pointing toward the
lumen. The function of the adult small intestinal epithelium is mediated by
four different types of mature cells: enterocytes, goblet, enteroendocrine and
Paneth cells. Undifferentiated cells reside in the crypts and produce these
four major types of mature cells. Enterocytes, goblet cells and enteroendocrine
cells migrate upwards along the crypt villus axis, while Paneth cells move

downward and are confined to the bottom of the crypts. Recently, genetic mouse studies have suggested several putative
stem cell markers such as Bmi1 and Lgr5. The niche-related Wnt, Bmp and Notch
signalling pathways have also been suggested as being involved in regulation,
lineage specification and the maintainance of the specific stem cell
microenvironment. In our laboratory, we isolated the first normal human
intestinal epithelial crypt (HIEC) cell model from the foetal human intestine
and in this study we show that HIEC cells, originally derived from the human foetal intestine, express a
number of cell markers previously reported in the intestinal stem cell
compartment such as BMI1, DCAMKL-1 and Musashi. We investigated the expression
of a panel of intestinal stem cell markers in HIEC cells under normal culture
parameters as well as under conditions that mimic the normal stem cell
microenvironment. The glycogen synthase kinase 3β (GSK-3β) inhibitor, SB-216763, as
well as R spondin-1 and Wnt3a, were used to activate the WNT/β-catenin-signaling pathway. Gene expression was
accessed by real-time polymerase chain reaction and Western blot. The results
showed that control HIEC express detectable levels of BMI1 and HOPX and low
amounts of LGR5 and PHLDA1 and, consistent with these observations, relatively
low β-catenin/TCF activity and high BMP activity was observed. Stimulation of
HIEC cells with R spondin-1 and Wnt3a induced β-catenin/TCF activity and
expression of the Wnt target genes, Cyclin D2 and LGR5. Dual activation of the
Wnt pathway and inhibition of the BMP pathway with noggin resulted in further
increases in LGR5 and PHLDA1 expression and a decrease in BMI1 and HOPX
cellular levels. Cell proliferation was not affected. These findings
demonstrate that established cultures of normal human intestinal epithelial
crypt cells can be prompted toward a stem-like cell phenotype by modulating a
few key cellular pathways. This model should be useful to further define the
human intestinal stem cell niche.

F-1106

THE PROSPECT OF STEM CELL RESEARCH AND DEVELOPMENT IN KOREA

Moo Woong Kim, Eun Jung Kim, Min Jung Kim, Younga Kim, Byuong Hwan Hyun
Korea Research Institute of Bioscience and Biotechnology, Daejoen, Korea, Republic of

Future health and medical technologies are expected to be centered on personalized prevention rather than on treatment. In particular, stem cell-related research and technologies are regarded as a major contributor to shift the paradigm of health and medical service technology in the 21st century. In this regard, the Korean government has been making every possible effort to establish various policies and systems that enables the promotion of stem cell research and development alongside the maintenance of regulations. In Korea's biotechnologies, stem cell technology has reached a relatively high level. With the technical prowess, biotech companies in Korea have been putting a lot of hard work into the development and commercialization of stem cell therapy products and, as a result 'HeartiCellgram-AMI (PharmiCell)' has been licensed in Korea in 2011. In addition, the U.S. FDA has allowed 'Cartistem (MediPost)' to be used as an investigational drug in a clinical trial. Herein lies the purpose to review the Korean government's hard work for making policies on stem cell research and development in addition to scientific and industrial outcomes, as well as to look into considerations about policies and regulations for sustainable stem cell research and development.

Other

F-1107

OPTIMIZING PUBLIC CORD BLOOD BANKING AND RESEARCH

Rosario Isasi, Bartha M. Knoppers
Centre of Genomics and Policy, McGill Univ, Montreal, QC, Canada

Umbilical cord blood (CB), long treated as waste material, is today considered a valuable source of multiple stem cells (SC) for both research and clinical applications. CB is currently regarded as standard practice for the treatment of hematologic diseases. The increase in the demand for CB for clinical applications, paired with the outstanding challenges for satisfying such demand (e.g. volume and expansion, HLA diversity, etc.), has generated, in turn, a surge in the use of CB for research. In addition to blood precursor cells, CB also contains SCs that can differentiate into other cell types fuelling speculation about the use of CB SCs for regenerative medicine. For instance, the derivation of human induced pluripotent SCs from CB cells suggests the potential for a valuable and affordable source for the development of novel therapies. Heterogeneous scientific practices (e.g. procurement) and socio-ethical and policy frameworks, together with the co-existence of public and private networks and institutions, have been identified as obstacles to equitable access.
In June 2013, Canadian Blood Services will establish and operate the national OneMatch Public Cord Blood Bank (OMPCBB) that will be accessible to Canadian and international patients and researchers. The OMPCBB will promote efforts that contribute to research and improved clinical care by making units not suitable for banking or transplantation available for research. In this context, the development of harmonized tools for UCB collection and uses that meets the needs of all stakeholders is critical. This is underscored by the fact that certain ethical and policy issues arising in the research context are distinct from those arising during the process of collecting, donating and using CB for banking and clinical applications such as transplantation.
In the context of the OneMatch Public Cord Blood Bank of the Canadian Blood Services , this presentation will focus on the practical tools (e.g. consent protocols) developed to optimize cord blood banking and research while enabling ethical provenance of UCB SCs. The Canadian model represents an ideal model for comparison as it is a country in which the national, public bank (and other regional public banks) co-exists with private companies.

Lung Cells

F-1111

EXAMINATION OF THE CANCER STEM CELL HYPOTHESIS IN HUMAN LUNG TUMORS

Kerstin Sinkevicius, Carla Kim
Boston Children's Hospital, Boston, MA, United States

Lung cancer is the major cause of death from cancer worldwide. The cancer stem cell (CSC) hypothesis, which suggests that tumors are maintained by a population of cells possessing stem cell characteristics, has emerged as an attractive explanation for tumor growth, recurrence, and metastasis. Although it has recently been shown that CD166 enriches for human lung cancer stem cells when primary uncultured human lung tumor cells were injected subcutaneously into immunocompromised NSG animals (Zhang et al), it will also be crucial to determine if CD166 marks a CSC population when the cells are injected into their normal lung microenvironment. I have determined

intravenous injections are the best orthotopic transplantation method to use to detect human lung CSCs. I sorted 12 human primary NSCLC tumor samples by FACS for CD24, CD133, or TrkB, and transplanted 100 to 10,000 cells by intratracheal injections into NSG mice. 2/12 and 0/12 mice transplanted with TrkB+ and TrkB- cells, 2/10 and 0/10 mice injected with CD133+ and CD133- cells, 2/8 and 1/8 mice injected with CD24+ and CD24- cells, and 0/9 CD271+ and 0/9 CD271- cells, respectively, developed tumors 1 year after transplantations. However, the tumors were very small and not large enough to sort again for serial transplantation. These results indicated intratracheal injections are not robust enough to use to detect a CSC population. I therefore injected several human NSCLC cell lines by various techniques to establish which injection method would lead to the best tumor formation. Cells were injected by either subcutaneous, intratracheal, tail vein, or ultrasound guided injections into the lungs of NSG mice and tumor burden was determined 3 months later. Mice injected by tail vein injections overwhelmingly developed the largest tumors. To establish if CD166 is a marker of human lung cancer stem cells in an orthotopic transplantation model, cells will be dissociated from human lung tumors using collagenase/dispase and FACS sorting will be used to exclude hematopoietic and endothelial cells using CD45 and CD31, respectively. The CD45-CD31- population will be further sorted for CD166 and transplanted by tail vein injections into NSG mouse recipients. Serial transplantations will be performed to examine self-renewal of the cells. If CD166 does not mark lung CSCs in these orthotopic assays, I will alternatively sort additional samples for other putative lung CSC markers including CD133, TrkB (which our lab has shown is critical for lung cancer metastasis), and CD24 (which our lab has shown marks a mouse metastatic-propagating cell population). If CD166 marks a human CSC population, CD166+ cells will be further sorted into TrkB+ and TrkB- and CD24+ and CD24- populations and transplanted by tail vein injections into NSG mice to determine if TrkB and/or CD24 mark metastatic-propagating CSCs.

F-1112

EFFECT OF NKX2.1 TRANSCRIPTION FACTOR HAPLOINSUFFICIENCY ON THE IN VITRO DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS INTO LUNG AND THYROID PROGENITORS

Maria Serra[1], Finn Hawkins[1], Mélanie Bilodeau[2], Ricardo H. Costa-E-Sousa[3], Janet Rossant[2], Anthony N. Hollenberg[3], Darrell N. Kotton[1]

[1]Pulmonary Center, Boston University, Boston, MA, United States, [2]Program in Developmental and Stem Cell Biology, Hospital for Sick Children, Toronto, ON, Canada, [3]Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, United States

The lung and thyroid epithelia derive from embryonic endoderm cells characterized by the expression of the transcription factor NKX2.1 (thyroid transcription factor-1 or TTF-1). NKX2.1 is also expressed in the developing forebrain. Despite its lack of specificity, NKX2.1 is the earliest known marker of lung and thyroid primordial cells and is used as a marker of specification of these lineages. NKX2.1 plays a crucial role in the development of the three tissues where it is expressed, driving morphogenetic processes and regulating the promoters of mature organ markers. Interestingly, mutations of NKX2.1 in humans cause the “Brain-Lung-Thyroid Syndrome” which causes severe brain disease in the form of choreoathetosis and also hypothyroidism and infant respiratory distress. The finding of heterozygous NKX2.1 loss of function mutations in some patients suggests NKX2.1 haploinsufficiency causes the disease.

The goal of our study is to use the in vitro embryonic stem cell system to model the earliest stages of lung and thyroid development and study the effect of Nkx2.1 haploinsufficiency in their specification and maturation. We engineered a mouse ES cell line with a GFP reporter targeted to the Nkx2.1 locus, allowing us to purify lung and thyroid Nkx2.1+ progenitors via definitive endoderm, using our published protocol for lung/thyroid directed differentiation. Because the targeting of the GFP reporter in this cell line disrupts the coding region of Nkx2.1, it can be used to model Nkx2.1 haploinsufficiency. In order to test the effect of Nkx2.1 haploinsufficiency on the differentiation of mESCs, we compared the Nkx2.1-GFP line to the parental line W4/129S6, as well as to a non-haploinsufficient Nkx2.1-mCherry knock-in line.

The efficiency of specification of lung and thyroid progenitors was estimated as the percentage of Nkx2.1 expressing cells, measured by intracellular FACS. No differences were detected when the Nkx2.1-GFP line was compared

to its parental line W4/129S6, both showing around 20% of cells expressing Nkx2.1. After expansion and differentiation, the expression of mature proximal (CC10) and distal (SPC) lung markers were detected by q-PCR at similar levels in the two lines. Interestingly, the expression of the thyroid markers Tg, TSH-R, NIS and TPO was significantly reduced in the haploinsufficient line. Next, we differentiated the Nkx2.1-GFP in parallel with the Nkx2.1-mCherry line. The Nkx2.1-GFP+ and Nkx2.1-mCherry+ progenitors were purified before they were further differentiated and the expression of lung and thyroid markers was analyzed. Again, a clear effect of happloinsufficiency on the expression of mature thyroid markers and in the early thyroid transcription factor Pax8 was detected, while the expression of mature lung markers SPC and CC10 was not affected significantly.
These preliminary results suggest that different threshold levels of Nkx2.1 expression may be required for the maturation of the lung and the thyroid lineages, and that Nkx2.1 deficiency may preferentially affect the maturation of the thyroid epithelium. These studies also show that the directed differentiation of mESC can be considered as a potential platform for in vitro modeling of disease caused by Nkx2.1 haploinsufficiency.

F-1113

IDENTIFICATION OF STEM /PROGENITOR CELLS AND THEIR NICHES IN HUMAN EMBRYONIC LUNG

Chava Rosen[1], Elias Shezen[1], Herzel Ben-Hur[2], Yair Reisner[1]
[1]immunology, Weizmann Inst of Science, Rehovot, Israel, [2]Obstetrics and Gynecology, Assaf Ha Rofe Hospital, Rishon le Zion, Israel

Mammalian lungs exhibit clear regenerative potential even after substantial damage. Presumably, the regenerative process in the lung involves resident stem cell populations. Recently, potential mechanisms of lung regeneration were identified in rodents, revealing involvement of typical embryonic signaling cascades. In the present study we attempted to identify stem and progenitor cell populations and their respective niches in human embryonic lung (HEL) tissues harvested at 20-22 weeks of gestation which are relatively enriched with respiratory structures compared to earlier gestational time points. Initial examination for the presence of basal epithelial lung progenitors, expressing cytokeratin 5 (CK5) and cytokeratin 14 (CK14), revealed $CK5^{+}CK14^{+}$ cells in the large airways, while single stained $CK5^{+}$ cells were more common in the developing alveoli. Since the functional distinction of $CK5^{+}$ from $CK5^{+}CK14^{+}$ cells is not yet fully understood, we focused our attention on the broader $CK5^{+}$ progenitors. Interestingly, these cells are commonly surrounded by $nestin^{+}$ cells, some of which exhibit properties of neuroepithelial bodies marked by calcitonin gene-related protein (CGRP). Epithelial progenitor innervation was further revealed by staining for neurofilaments (NF). This unique structure of progenitor cells surrounded by innervating cells suggests a stem cell niche architecture, similar to that previously defined for hematopoietic stem cells in the bone marrow and in adult mouse airways. In addition, in line with a recent report regarding the bone marrow niche, the epithelial $CK5^{+}$ niche also contained alpha-smooth muscle actin positive cells and $vimentin^{+}$ mesenchymal cells.
Polychromatic FACS analysis of single cell preparations of HEL revealed three distinct non-hematopoietic $CD45^{-}C\text{-}Kit^{+}$ (CD117) progenitor sub-populations, including $CD34^{high}$, $CD34^{+intermediate}$ and $CD34^{negative}$ cells. While the $CD34^{negative}$ population is compatible with the early pluripotent adult lung stem cells recently described by Kajstura et al., (NEJM, 2011) the other $CD34^{+}$ cells, also expressing high levels of CD31, might be more strongly committed towards the endothelial lineage. Importantly, the $C\text{-}Kit^{+}CD34^{neg}$ subpopulation, also negative for CD271 (NGFR- mesenchymal stem cell marker) and CD326 (EPCAM- epithelial differentiation marker), was clearly more abundant in HEL tissues harvested at 20 weeks (up to 2-3% of $CD34^{neg}$ population) compared to adult lung tissue used as a control (<0.45%).
Interestingly, immune-histological staining showed that the majority of CD117+ cells co-expressed CD34 and resided in blood vessel walls, surrounding developing alveoli, while the minor unique CD117+ single positive cell sub-population was found in close proximity to large blood vessels and large airways. Furthermore, HEL tissues also exhibited early (CD45+/CD14+) and late (CD45 - /CD105 +) endothelial progenitor cells (EPC).

Taken together, our demonstration that HEL exhibit high level of distinct progenitor cell populations within unique niches, strongly suggests that HEL could potentially offer a novel rich source for lung repair .

F-1114

REDOX REGULATION OF AIRWAY EPITHELIAL STEM CELL MAINTENANCE AND REPAIR

Bharti Bisht, Manash Paul, Daphne Darmawan, Richard Chiou, Andy Chon, Tristan Grogan, Jackelyn Alva, David Elashoff, Brigitte Gomperts
Mattel Children's Hospital UCLA, Los Angeles, CA, United States

The epithelial lining of the conducting airways of the lungs is the first line of defense against inhaled particles and pathogens that can cause injury. Airway epithelial stem cells (SCs) play a vital role in repairing the epithelial barrier after injury through a tightly regulated process that prevents excessive proliferation. However, this process is poorly understood.
Although historically viewed as harmful, recent evidence suggests that reactive oxygen species (ROS) function as important physiological regulators of intracellular signaling pathways. Several studies have identified the role of ROS in stem cell maintenance, repair and tissue regeneration. However, there has been controversy in the field as to whether stem cells have high or low ROS levels. We hypothesized that ROS may play a role in regulating repair from airway epithelial SCs.
We sorted airway epithelial SCs on the basis of their ROS levels and cultured them in the in vitro sphere-forming assay. We found that ROS low populations of SCs were highly enriched for sphere-forming ability. Using the in vitro sphere-forming assay as a measure of stem cell proliferation, we manipulated ROS levels by adding exogenous ROS with hydrogen peroxide (H2O2) or scavenged ROS with N-acetyl cysteine (NAC) and found that an increase in ROS from low to intermediate levels (“sweet spot”) of ROS is most effective at promoting proliferation and levels above or below this greatly reduce the ability of these SCs to proliferate. We also looked at ROS in individual SCs using the FUCCI cell cycle tracker. We found that quiescent SCs are predominantly low in ROS and that ROS increases during the G1 to S transition and then decreases during mitosis to basal levels.
The primary cellular defense against oxidative stress is the transcription factor, Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which activates a number of responses to ROS within a cell. Nrf2-/- mouse SCs express high levels of ROS and do not form spheres in culture, but can be partially rescued with NAC. We found that administration of an airway epithelial injury to Nrf2-/- mice results in severely delayed repair of the epithelium with reduced proliferation as compared to injured wild type airway.
Gene expression profiling of stem versus non-stem cells led us to examine the Notch pathway for airway epithelial repair. Prior work has shown the importance of Notch in airway epithelial cell differentiation but we demonstrated a role for Notch in proliferation of SCs using sphere and air-liquid interface assays with Notch activation and inhibition prior to differentiation. Furthermore, airway epithelial injury in transgenic mice with activation of Notch in their SCs results in hyperplasia of the airway epithelium, which is not seen in wild type mice.
We hypothesized that Nrf2 regulates Notch expression and that Notch is a downstream effector of ROS flux. We used EMSA to demonstrate that Nrf2 binds to an ARE binding site in the Notch promoter in airway epithelial SCs. Notch reporter studies demonstrated that flux of ROS levels from low to the “sweet spot” level in SCs increases Notch expression. Furthermore, stabilization of Nrf2 with sulforaphane also increased Notch expression. We have identified a ROS-Nrf2-Notch-dependent mechanism that tightly controls SC proliferation in airway epithelial repair.

Epidermal Cells

F-1121

RUNX1 CONTROLS A REVERSIBLE TRANSITORY STATE BETWEEN STEM AND PROGENITOR CELLS IN MOUSE HAIR FOLLICLE THAT IS AT THE ORIGIN OF SKIN TUMORS

Tudorita Tumbar
Molecular Biology and Genetics, Cornell University, Ithaca, NY, United States

Our lineage tracing experiments in mice demonstrate that Runx1/AML1 marks the long-term stem cells (SC) of the hair follicle and oral epithelium, and Runx1+ cells are at the origin of skin epithelial tumors. Runx1 is necessary for hair follicle bulge SC activation and for skin and oral tumor formation and maintenance in mice. Runx1 is also indispensable for skin and oral human squamous cell carcinoma growth in culture. Quantification of Runx1 endogenous expression levels in hair follicle revealed large variations with hair cycle stage and cell type. We employed a tetracycline inducible system in mice to manipulate Runx1 expression in vivo. In progenitor hair germ and matrix cells, elevated Runx1 levels increase the expression of some bulge SC markers, block further differentiation and cause unexpected apoptosis and premature hair regression (catagen) followed by permanent arrest (telogen). Conversely, in bulge SCs elevated Runx1 levels result in progression to progenitor/transit amplifying (TA) cells, characterized by an initial increase of proliferation, down-regulation of many bulge SC markers and up-regulation of some germ cell markers. Eventually these bulge cells cease proliferation, senesce and die, which result in permanent hair loss. The SC to TA cell conversion is reversible, because withdrawal of Runx1 expression after a period sufficient to cause the conversion, results in return of hair follicles to normal. Loss of p53 rescues the cell death phenotype of Runx1 over-expressing cells in culture, while in tissue the Runx1-high; p53-/- follicle cells are cleared by inflammation. We propose that high Runx1 levels mark an intermediate, short-lived, and reversible SCs to TA transitory state, which is essential for normal SC activation in early hair cycle. Maintenance of this state in proliferative cells is not tolerated, and in fact Runx1-elevation is utilized latter in hair cycle for regression (catagen). We further propose that this unstable and reversible state between SC and TA cells is the true origin of tumors and it becomes stabilized by simultaneous loss of p53 and evasion of the immune system, resulting in neoplastic growth and long-term tumor cell maintenance.

F-1123

MOLECULAR MECHANISMS THAT CONTROL KERATINOCYTE SPECIFICATION

Ana Tadeu, Valerie Horsley
MCDB, Yale University, New Haven, CT, United States

While the molecular mechanisms that drive the differentiation of basal keratinocytes into suprabasal cells during development are known, the earliest stages of specification of keratinocytes from non-neural ectoderm during development are not well defined. Here, we use both developing mouse embryos and human embryonic stem cells to explore the mechanisms that direct keratinocyte fate from ectodermal progenitor cells. We show that both human embryonic stem cells (hESCs) and murine embryos express DeltaNp63 prior to keratin 14 (K14) expression. Furthermore, we find that Notch signaling is activated prior to DeltaNp63 expression in ectodermal progenitor cells and is repressed upon DeltaNp63 expression. Pharmacological and genetic inhibition of Notch signaling reveals a negative regulatory role for Notch signaling in p63 expression during ectodermal specification. Taken together, these data reveal a role for Notch signaling in the molecular control of ectodermal progenitor cells to the keratinocyte lineage. These data suggest that manipulation of Notch signaling during differentiation of hESCs could promote efficient generation of keratinocytes for cell therapies for skin burns, wounds and genetic abnormalities.

F-1124

LOCALIZATION OF P63 AND P63 PHOSPHORYLATION IN EQUINE (EQUUS CABALLUS) EPIDERMAL TISSUES: POTENTIAL MARKERS OF EPIDERMAL STEM CELLS AND THE TRANSITION TO TRANSIT-AMPLIFYING CELLS

Renata Linardi, Hannah Galantino-Homer, Makoto Senoo
UPENN, Kennett Square, PA, United States

Localization of p63 and p63 phosphorylation in equine (Equus caballus) epidermal tissues: Potential markers of epidermal stem cells and the transition to transit-amplifying cells**.**
Renata L. Linardi, University of Pennsylvania School of Veterinary Medicine, Department of Clinical Studies/New Bolton Center,
382 West Street Rd

Kennett Square, PA 19348-1692
rlinardi@vet.upenn.edu
610-925-6204
Makoto Senoo, University of Pennsylvania School of Veterinary Medicine, Department of Animal Biology, Philadelphia, PA, msenoo@vet.upenn.edu
Hannah L. Galantino-Homer, University of Pennsylvania School of Veterinary Medicine, Department of Clinical Studies/New Bolton Center, hghomer@vet.upenn.edu
Equine laminitis is a severe, common and debilitating disease of horses' feet characterized by inflammation, damage, and dysplasia of epidermal and dermal lamellae which attach the hoof to the underlying distal phalanx bone. We have demonstrated previously that chronic laminitis results in the generation of dysplastic epidermal lamellae that are deficient in the epithelial transcription factor and marker of epidermal stem cell (ESC) proliferative potential, p63. However, p63 expression extends into the suprabasal cell layers of equine epidermal tissues and may not exclusively identify ESCs since the ESC niche is believed to be restricted to the basal compartment in stratified epithelia. Our hypothesis is that p63 is highly expressed in equine ESCs and that concurrent serine 160/162 p63 phosphorylation (P-p63) marks the transition from ESCs to transit-amplifying (TA) cells. Tissues of the eye (limbus, and cornea) and foot (skin, coronary, and hoof lamellae) were harvested immediately post mortem and either snap frozen for immunoblotting (IB) or fixed in 10% formalin and paraffin-embedded for indirect immunofluorescence (IIF) analysis, both performed using a mouse monoclonal against human p63 (clone 4A4) or a rabbit polyclonal against P-p63 (clone Ser 160/162). IB revealed a protein band of the appropriate relative molecular mass in all tissues of the eye, foot and adjacent skin. IIF studies demonstrated that p63 was expressed in the nuclei of a subset of basal cells and with decreasing intensity in the suprabasal cells of equine skin, coronary, lamellae, limbal, and corneal epidermal tissues. Cells with nuclear expression of P-p63 were also localized to an overlapping subset of both basal and suprabasal layers of the tissues, becoming more apparent as p63 immunolocalization decreases with distance from the basal layer and with increasing epidermal differentiation. A subset of basal cells was p63-positive/P-p63-negative and might represent ESCs. Conversely, the subset of suprabasal cells that is P-p63-positive/p63-negative might represent the terminally differentiating cells while the cells that co-localize both markers could be TA cells. These data support, but do not prove, our hypothesis that p63 phosphorylation marks the transition from ESC to TA cells. Investigation of the in vitro clonogenic potential of p63-positive vs P-p63-positive cell populations is needed to more thoroughly determine the ESC proliferative potential of these two cell populations. To our knowledge, this is the first report of P-p63 localization in equine epidermal tissues and its potential use as a marker for initial epidermal cell differentiation.
Key words: Laminitis, Epithelial stem cell, Epidermal lamellar cell, Keratinocytes.

F-1125

CELL DENSITY-DEPENDENT UPREGULATION OF PDCD4 IN KERATINOCYTES AND ITS IMPLICATION FOR EPIDERMAL HOMEOSTASIS AND REPAIR

Wang Tao[1], Su Yongping[1], Zou Zhongmin[2], Shi Chunmeng[1], Wang Junping[1], Li Rong[1], Ran Xinze[1]
[A]Institute of Combined Injury,State Key Laboratory of Trauma, Burn and Combined Injury, [B]Department of Chemical Defense and Toxicology, [1]College of Preventive Medicine,Third Military Medical University, Chongqing, China

Cutaneous wound healing is a complicated process involving inflammation, cell migration, proliferation, and tissue remolding. It's known to all that both wounds and cancer tissue have highlighted remarkable similarities with common cellular and molecular mechanisims. Upon skin injury, wound activates cancer-provoking genes and the repair cells exhibits a similar behavior like cancer cells, which are critical for normal wound healing. However, in contrast to cancer tissue, the process of wound healing is self-limiting, resulting in controlled cell proliferation, differentiation, migration and remodeling. Logically, the self-limiting of wound healing is partly due to the function of tumor suppressor genes. While the role of tumor suppressors in epidermal homeostasis and repair is still unclear. Here we show that the new tumor suppressor programmed cell death 4 (PDCD4) is induced in a cell density-dependent manner in HaCaT keratinocyte cells and this upregulation of PDCD4 protein is in accord with induced mRNA

level. To determine the potential role of PDCD4 in keratinocyte cell biology, we show that knockdown of PDCD4 by two siRNAs can promote cell proliferation in lower cell density and partially impair contact inhibition in confluent HaCaT cells. In normal mouse skin tissue, PDCD4 is expressed mainly in hair follicle, sweat gland and interfollicular epidermis and displays diffuse cytoplasmic staining. Meanwhile, we find that there is significantly enhanced expression of PDCD4 in anagen hair follicle than that in telogen hair follicle and there is mutual inverse expression of PDCD4 and Ki67 in the anagen hair follicle. Then, the expression pattern of PDCD4 in repaired epidermis is examined using excision wound model. We find that PDCD4 is reduced in the activated migratory keratinocytes during reepithelialization. While, enhanced nucleus-staining PDCD4 is detected in the hyperproliferative wound epidermis as early as 7-day wound. Both the enhanced nucleus and cytoplasm staining of PDCD4 are observed in the epidermal maturation stage of reepithelialization in 16-day and 25-day wounds. Thus, our results suggest that tumor suppressor PDCD4 is uniquely induced in a cell density-dependent manner in keratinocyte cells and serves as important regulator of keratinocyte cell proliferation and contact inhibition i*n vitro*. And what's more, enhanced expression of PDCD4 is detected in both anagen hair follicle and transient hyperproliferative wound epidermis *in vivo*, which suggests the important steady-state regulating role of PDCD4 in epidermal homeostasis and wound healing.

F-1126

ROLES OF WWP2 AND LSD1 IN MULTIPOTENT MURINE SKIN PRECURSOR CELLS

Sang Kyu Park, Sangho Roh
Lab of Embryo Biotechnology and Cellular Reprogramming, School of Dentistry, Dental Research Institute, Seoul National University, Seoul, Korea, Republic of

Multipotent skin-derived
precursor cells (SKPs) are easily obtained from the skin. In addition to their merit as good source for cell therapy, SKPs can be a useful tool for the
research of embryonic development as they include neural crest cell population. Atrophin-1-interacting protein 2 (WWP2) is an E3 ubiquitin ligase, which is related to transcription for embryonic stem cell fate control. Lysine-specific demethylase 1 (LSD1) plays an important role in embryonic development and also in the maintenance of pluripotency in embryonic stem cells. Here we discovered the role of WWP2 and LSD1 in SKPs. The cells from murine back skin dermis were propagated after dissociation into single cells by the treatment of trypsin, dispase and collagenase in DMEM-F12 (3:1) containing B27 supplement (2%), EGF (20 ng/ml) and FGF (40 ng/ml). Putative SKPs were passaged in the medium supplemented with accutase. Then, the cells were induced neurogenic, adipogenic and myogenic lineage differentiation by standardized protocols, which are properties of SKPs. The cells had abilities of neurogenic, adipogenic and myogenic differentiation and were confirmed to be SKPs. We analyzed the expressions of WWP2, LSD1, pluripotency-, and differentiation-related genes in SKPs. In addition, the properties of senescence, differentiation and sphere forming potential, which can be determined by the clonogenicity and the size of the sphere, were investigated after downregulation of WWP2 and LSD1 using 7 WWP2 and 5 LSD1 miRNAs. The downregulation of WWP2 and LSD1 tended to change the properties aforementioned. Detailed results will be presented at the meeting. The results reveal that WWP2 and LSD1 may have an important role in SKPs in terms of their differentiation and/or senescence. This study was supported by a grant from the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (MEST; 2012-0006145) and Technology Development Program for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry and Fisheries (MIFAFF; 111160-04), Republic of Korea.

F-1127

SOX2 MODULATES THE FUNCTION OF TWO DISTINCT CELL LINEAGES IN MOUSE SKIN.

Marta H. Lesko[1], Ryan R. Driskell[1], Kai Kretzschmar[1], Stephen Goldie[2], Fiona M. Watt[1]
[1]Centre for Stem Cells and Regenerative Medicine, King's College London, London, United Kingdom, [2]Cambridge Research Institute, University of Cambridge, Cambridge, United Kingdom

In postnatal skin the transcription factor Sox2 is expressed in the dermal papilla (DP) of guard/awl/auchene hair follicles and by mechanosensory Merkel cells in the touch domes of Guard hairs. Sox2 is also a marker of multi-potent skin progenitor cells (SKPs). To investigate the consequences of Sox2 ablation in skin we deleted Sox2 in DP cells via Blimp1Cre and in Merkel cells via K14Cre. Loss of Sox2 from the DP did not inhibit hair follicle morphogenesis or establishment of the dermis and hypodermis. However, Sox2 expression in the DP was necessary for postnatal maintenance of awl/auchene hair follicles. Deletion of Sox2 via K14Cre resulted in a decreased number of Merkel cells but had no effect on other epithelial compartments or on the dermis. The reduced number of Merkel cells did not affect the number or patterning of guard hairs, nerve density or the interaction of nerve cells with the touch domes. We conclude that Sox2 is a marker of two distinct lineages in the skin and regulates the number of differentiated cells in the case of the Merkel cell lineage and differentiated function in the case of the DP.

F-1128

PERIVASCULAR HAIR FOLLICLE STEM CELLS ASSOCIATE WITH A VENULE ANNULUS

Ying Xiao, Isaac Brownell
NIH, Bethesda, MD, United States

Maintenance of stemness is especially important for hair follicle stem cells because follicles are highly proliferative and continually cycle through phases of regression and stem cell mediated regeneration. In addition to trophic support, vasculature is involved in regulating many processes including the creation of microenvironments that maintain stem cells in some tissues. We sought to investigate if vasculature also provides a hair follicle stem cell niche. We found that dermal blood vessels form a loop around K15- hair follicle stem cells in the upper bulge. This vascular annulus consistently remains associated with the K15- region despite extensive angiogenesis and remodeling during hair follicle cycling. Using the venous marker EphB4, we discovered the vascular annulus is comprised of post-capillary venules, suggesting the important notion that relative hypoxia and other venous traits might contribute to the stem cell niche. Intriguingly, the K15- upper bulge specifically expresses Egfl6, a signaling molecule with angiogenic properties that can influence endothelial cell migration, suggesting a mechanism for maintaining its association with the vascular annulus. Developmentally, we found that nascent hair placodes begin recruiting blood vessels by embryonic day E14.5, and a vascular annulus forms by birth when K15- upper bulge cells are specified. In altered folliculogenesis, a stereotypical vascular annulus is recapitulated around the upper bulge of de novo reconstituted hair follicles. The consistent and persistent nature of this association in different contexts strongly suggests a necessary regulatory relationship between the perivascular microenvironment and K15- upper bulge stem cells. We propose that specialized hair follicle stem cells are associated with a venule vascular niche that may be instrumental in establishing and maintaining tissue specific stem cell. Identifying niche components for normal hair follicle stem cells should help in understanding the niche for basal cell carcinoma cancer stem cell, which might originate from hair follicle stem cells.

Epithelial Cells (Not Skin)

F-1131

REGULATION OF AIRWAY BASAL STEM/PROGENITOR CELLS BY AMPHIREGULIN

Wu-Lin Zuo, Renat Shaykhiev, IonWa Chao, Ronald G. Crystal
Genetic Medicine, Weill Cornell Medical College, New York, NY, United States

Airway epithelium is a pseudostratified layer composed of 4 major cell types, including ciliated and secretory cells, columnar undifferentiated cells, and basal cells (BC). BC are stem/progenitor cells of the airway epithelium, capable of self-renewal and differentiating into ciliated and secretory cells. Based on the knowledge that BC express epidermal growth factor receptor (EGFR), and amphiregulin (AREG), one of the EGFR ligands, is expressed in human T cells, we hypothesized that AREG expressed by airway-resident T cells within the BC microenvironment regulates BC stem/progenitor cell functions. Immunofluorescence analysis of biopsy samples of normal human airway epi-

thelium revealed that AREG was expressed by intraepithelial and subepithelial T cells located in close proximity to BC. To study the effects of AREG on BC functions, BC were isolated from human airway epithelium obtained by bronchoscopic brushing and recombinant human AREG (10 ng/ml) was added to BC during differentiation in air-liquid interface (ALI). Airway epithelium generated during 28 days of ALI from AREG treated BC had increased expression of proliferation marker Ki67 as determined by Taqman PCR analysis, accompanied by increased number of Ki67-positive BC compared to the control group. Exposure of BC to AREG shifted the differentiation of BC towards the mucous-producing phenotype with up-regulation of goblet/mucous cell makers MUC5AC, MUC5B, TFF3, SPDEF and AGR2. AREG-induced expression of goblet cell marker TFF3 was reduced by application of Notch inhibitors (γ-secretase inhibitors DBZ and DAPT). Increased proliferation and goblet/mucous cell differentiation in airway epithelium derived from AREG-treated BC was paralleled by decreased expression of the ciliated cell markers FOXJ1, DNAI1, IFT172 and suppression of the airway epithelial barrier integrity and function as determined by transepithelial resistance decrease, down-regulation of junctional genes TJP1, TJP3, PARD3, PARD6B, CLDN3 and increased paracellular flux of FITC-dextran through the epithelial layer. In summary, AREG expressed by T cells represents a microenvironmental signal mediating BC interaction with T cells and regulating BC stem/progenitor cell function by inducing BC proliferation and mucous cell differentiation, suppressing ciliated cell differentiation and decreasing the epithelial barrier integrity. In the context of these observations, AREG represents a signal capable of driving the airway epithelium toward the BC/secretory cell hyperplastic phenotype commonly observed in airway disorders.

F-1132

CORRECTION OF METABOLIC LIVER DISEASE AND ACUTE LIVER FAILURE BY THE TRANSPLANTATION OF HUMAN AMNION EPITHELIAL STEM CELLS.

Stephen C. Strom[1], Kristen J. Skvorak[2], Veysel Tahan[2], Kenneth Dorko[2], Roberto Gramignoli[1]

[1]Laboratory Medicine, Karolinska Intitutet, Stockholm, Sweden, [2]Department of Pathology, University of Pittsburgh, Pittsburgh, PA, United States

Background. Our laboratory has promoted the translation of hepatocyte transplant technology from the bench to the clinic. However, a severe shortage of useful hepatocytes limits a wider application of this cellular therapy. Stem cell sources are being investigated for the production of cells for transplants. Human amniotic epithelial (hAE) cells have been shown to have gene expression profiles and surface markers characteristic of pluripotent stem cells. They are not tumorigenic and when transplanted into the liver of mice, express human liver genes including CYP450, phase II, hepatic transporter and nuclear hormone receptor genes at levels observed in normal adult human liver. In addition, hAE have anti-inflammatory and immunomodulatory properties that may be beneficial in transplant patients. Because of these diverse properties, we proposed that hAE could be useful for the treatment of metabolic liver disease or acute liver failure (ALF). Aim: We evaluated the possible beneficial effects of hAE transplants in a mouse model of ALF and 2 mouse models of metabolic liver disease; intermediate Maple Syrup Urine Disease (iMSUD) and Phenylketonuria (PKU). Materials and Methods. ALF was induced in C57/Bl6 mice by d-galactosamine (d-gal; 5g/kg) IP. Six hours later groups of 6 mice each received 2x106 hAE cells via splenic injection or saline as control. Mice with PKU or iMSUD received two direct hepatic injections of 1x106 hAE cells during the first 10 days of life (DOL), followed by two additional hepatic transplants (2x106 cells each) between days 21-35 DOL. Blood and tissues were analyzed at 35 and 100 DOL. Results. Acute Liver Failure: Animals that received d-gal developed severe liver failure and all untreated or saline treated animals died within an average of 2.2 days with significant increases in AST, ALT and TGF-beta levels, and liver pathology consistent with ALF. All d-gal-intoxicated animals that received hAE cell transplants survived until scheduled sacrifice at day 14. In separate experiments with animals sacrificed at day 3 post d-gal treatment, transplant of hAE cells significantly decreased serum AST, ALT, and TGF-beta levels and decreased hepatic expression of Interleukin 1-beta, and Tumor Necrosis Factor alpha, and increased IL-10 expression. Metabolic liver diseases: All iMSUD-affected animals that did not receive hAE transplants died prior to day 35. Over 80% of hAE treated animals survived to sacrifice at 100 days with normal weight gain and behavior. Transplants of hAE significantly increased branched chain alpha-keto acid dehydrogenase (BCKDH) enzyme activity resulting in significantly improved levels of branched chain amino acids; leucine, isoleucine, valine

and alloisolucine and other large neutral amino acids in sera and brain. The amino acid neurotransmitter taurine and its precursors cysteine and glycine were normalized, as was gamma-aminobutyric acid (GABA) and serotonin levels. Both serotonin and dopamine turnover was partially corrected. In preliminary studies with PKU animals, hAE transplants normalized serum Phenylalanine levels. Conclusions. Human AE transplants prevent acute liver failure and also improve survival and correct many of the amino acid and neurotransmitter abnormalities observed in mice with metabolic liver disease. These successful pre-clinical studies have motivated translation to GMP isolation and banking of hAE at our Institutet for the cellular therapy for liver disease.

F-1133

BMI1-MEDIATED REPRESSION OF INK4A/ARF AND HOX GENES CONTROLS STEM CELLS IN THE RODENT INCISOR

Jimmy Hu[1], Brian Biehs[1], Nicolas Strauli[2], Eugenio Sangiorgi[3], Alice Goodwin[1], Sunita Ho[2], Mario Capecchi[3], Ophir Klein[1]

[A]Orofaical Sciences, [1]UCSF, San Francisco, CA, United States, [2]University of Utah, School of Medicine, Salt Lake City, UT, United States

The polycomb group gene Bmi1 is required in many organs, including the haematopoietic and nervous systems, for maintenance of adult stem cells. Inactivation of Bmi1 leads to de-regulated gene expression, resulting in impaired stem cell self-renewal because of cell cycle arrest. One critical target of Bmi1 is the Ink4a/Arf locus, which encodes the cell cycle inhibitors p16ink4a and p19Arf. Deletion of Ink4a/Arf partially rescues loss of Bmi1 function. However, Bmi1/Ink4a/Arf triple mutants are not completely normal, indicating that other important targets of Bmi1 remain to be studied. Here, using the continuously-growing mouse incisor as a model system, we report that Bmi1 is expressed by incisor stem cells and that deletion of Bmi1 results in fewer stem cells, change in stem cell identify, and defective production of enamel. While removal of Ink4a/Arf in the Bmi1 null background was able to restore the ability of insicor stem cells to self-renew, it could not rescue other phenotypes. Transcriptional profiling and functional assays revealed that Hox gene expression is normally repressed by Bmi1 in the adult to preserve the undifferentiated state of stem cells. Using an in vitro system, where primary incisor stem cells can be maintained and expanded in culture, we were able to demonstrate that Hox gene upregulation in Bmi1 mutants mediates changes in stem cell identity, as shRNAs against Hoxa9/c9 was able to rescue the phenotype of cells derived from Bmi1/Ink4a/Arf triple mutants. Thus, our findings point to a general mechanism whereby Bmi1-mediated repression of Hox genes is required for the maintenance of adult stem cells and for prevention of inappropriate lineage decisions by their progeny.

F-1134

ROLE OF ALDH- CELLS IN AN ORTHOTOPIC CANCER STEM CELL MOUSE MODEL

Sudha Krishnamurthy, Mitchell J. Frederick, Jeffrey N. Myers
Head and Neck Surgery, UT MD Anderson Cancer Center, Houston, TX, United States

Background: Head and Neck Cancer Stem Cells (HNCSC) comprise a small sub-population of cells with increased tumorigenic potential and resistance to conventional therapy. Aldehyde Dehydrogenase activity (ALDH+) has been used as a means of identifying and isolating these HNCSC. However, the role of the non-tumorigenic cells or non-cancer stem cells in tumor growth and progression has not been well elucidated.
Objective: To evaluate the role of tumorigenic (ALDH+) and non-tumorigenic (ALDH-) cells in tumor progression in an orthotopic mouse model of head and neck squamous cell carcinomas.
Methods: ALDH+ and ALDH-cells were isolated using Fluorescence Activated Cell Sorting from a panel of 4 different Head and neck squamous carcinoma cell lines; (n=10) and injected in the tongues of nude adult mice. Tumor xenografts were processed for histologic examination and percentage of cancer stem cells were analyzed. Stably generated fluorescent cells enabled tracking ALDH+ and ALDH- cells over time both *in vitro* and *in vivo* and study their fate.

Results: ALDH+ cells were more tumorigenic than ALDH- cells in four different head and neck cancer cell lines at a statistically significant rate ($p<0.05$). ALDH+ tumor xenografts had a high frequency of lymph nodal metastases of 33.5% (+/- 6.25%) in comparison to the ALDH- tumor xenografts (3.125%; $p<0.05$). Interestingly, there were distant metastases to the lung found in 22.22% of the ALDH+ xenografts in comparison to none in the ALDH- xenografts (n=10). Trying to understand the long term fate of ALDH- cells in tumor growth and progression, we injected a combination of ALDH+ cells and ALDH- cells with fluorescent labels, and observed that the 70% of the tumor cell population are of ALDH+ origin. However, the ALDH- cells survived over the entire period of 60 days and comprised about 30% of the tumor cell population. A similar representation of the cellular dynamics of the ALDH+ and ALDH- was seen *in vitro* as well; suggesting a long term proliferative potential in some of the ALDH- cells.
Conclusions: Orthotopic tongue tumors works as a good model system to study cancer stem cell initiation, growth and progression in head and neck cancers. ALDH+ cells were not only more tumorigenic than ALDH- cells, but were also regionally and distantly metastatic to the lungs in this model system. Though the cell population heterogeneity is predominantly established by ALDH+ cells and its derivatives, a sub-population of the ALDH- cells comprises a significant percent *in vitro* and *in vivo* suggesting the potential of long term proliferation.

F-1135

FABRICATION OF CORNEAL EPITHELIAL CELL SHEETS MAINTAINING EPITHELIAL STEM CELLS WITHOUT FEEDER CELLS BY OXYGEN-CONTROLLED METHOD

Ryota Nakajima, Shizu Takeda
Central Research Laboratory, Hitachi, Ltd., Saitama, Japan

Autologous corneal epithelial cell sheets have been utilized for treatment of ocular surface diseases such as chemical and thermal burns or Stevens-Johnson syndrome, which severely damages the limbus. Although murine 3T3 feeder cells have been used for ex vivo expansion of corneal limbal epithelial cells conventionally, the use of 3T3 feeder cells needs to be avoided to prevent xenogeneic infection and contamination. However, the expression level of epithelial stem/progenitor cell maker, p63, is down-regulated in case of a feeder-free culture system. The existence of p63-positive cells in the graft is important to succeed long-term corneal regeneration. In the present study, in order to fabricate the corneal epithelial cell sheets maintaining stem cells without feeder cells, we applied the oxygen-controlled method which was developed previously for efficient fabrication of cell sheets. Rabbit corneal limbal epithelial cells were cultured under hypoxia (1-10% O2) to reach confluence and then cultured under normoxia (20% O2) during stratification with and without 3T3 feeder cells. Multilayered corneal epithelial cell sheets were fabricated by the oxygen-controlled method and immunofluorecence analysis showed that cytokeratin 3 and p63 were expressed in appropriate localization of the cell sheets. On the other hand, colony-forming efficiency of the cell sheets fabricated by the oxygen-controlled method without feeder cells was significantly higher than that fabricated under 20% O2 without feeder cells. These results indicate that the oxygen-controlled method is effective to fabricate stratified epithelial cell sheets maintaining epithelial stem cells in a feeder-free culture system for corneal regenerative medicine.

F-1136

LONG TERM CULTURE OF CORNEAL EPITHELIAL PROGENITOR CELLS

Shigeto Shimmura, Hideyuki Miyashita, Satoru Yoshida, Tetsuya Kawakita, Kazuo Tsubota
Keio Univ Sch of Medicine, Tokyo, Japan

Corneal epithelial stem cells are located in the limbus, the junction between cornea and conjunctiva. Cultivated corneal epithelial cell sheets are used for ocular surface reconstruction of limbal stem cell deficiency patients. In this study, we devised a cultured medium containing KGF and the Rho kinase inhibitor Y-27632 that can be used to culture stratified limbal epithelial cell sheets for up to 3 months *in vitro*. To culture cell sheets, epithelial cells were isolated from human limbal tissue, seeded in cell culture inserts, and co-cultured with human mesenchymal stem cell-derived feeder cells. Immunohistochemistry showed the expressions of progenitor cell markers p63 and

cytokeratin 15 in the basal layer, and corneal epithelium specific differentiation markers cytokeratin 3 and cytokeratin 12 in supra-basal layers in both 1 month and 3 month cultured sheets. In addition, colony forming efficiency did not change between 1 and 3 months (22.3%±16.0%, n=4 and 23.3% ± 7.3%, n=5, p = 0.90, Student's t test). These results suggest that our sheets maintain corneal progenitor cells for at least 3 months in culture. Long term maintenance of cell sheets without impairment in progenitor cell population will enable a flexible schedule for transplantation.

F-1137

PROGENITOR BASAL CELL DYNAMICS OF THE MURINE NASAL CAVITY

Dawn T. Bravo[1], Ethan Soudry[1], Alan Nguyen[1], Wei Le[1], Justin Edward[1], Russel B. Fletcher[2], John Ngai[2], Jayakar V. Nayak[1]

[1]Otolaryngology Head &Neck Surgery, Stanford University School of Medicine, Stanford, CA, United States, [2]Molecular and Cell Biology, Helen Wills Neuroscience Institute, University of California, Berkeley, Berkeley, CA, United States

The p63 transcription factor has been shown to be a key regulator of epithelial stem cells, functioning to maintain self-renewing proliferative capacity and epithelial differentiation events. The central role for p63 in epithelial regeneration led us to determine whether p63-positive cell populations are present in the epithelium of the nasal cavity upper airway.

Studies of stem cell populations within the lower airways have established cytokeratin 5 (Krt5)+/p63+ basal cell populations as the likely pool of reserve cells involved in pulmonary self-renewal. Additionally, in olfactory epithelium, p63 serves a role in maintaining olfactory stem cells in a quiescent, undifferentiated, self-renewing precursor state.

Here we have identified Krt5+/p63+ cells as an uncharacterized population of basal cells present in the murine nasal airway epithelium. We analyzed the fate of these cells from embryonic (E) mice ages through advanced postnatal (P) ages. Unexpectedly, we find discrete recesses that are highly enriched for basal cell populations, and are devoid of differentiated cells. Furthermore, these recesses were found to be highly proliferative centers, especially along the outer periphery. These cells also co-express the nerve growth factor receptor (NGFR) basal cell marker. As mice aged, the presence of proliferative activity was significantly reduced compared to younger mice. Additionally, towards the end of the lifespan of the mouse, the number of p63+ basal cells decreased in number dramatically.

The results presented here indicate potential nasal cavity niche areas that are abundant in Krt5+/p63+ basal cells. These sites potentially supply the nasal respiratory epithelium with nascent basal cells. Two populations of basal cells are present; those that are proliferative and another that may be quiescent. The data also identify Krt5+/p63+ basal cells that change with aging. Future work will assess the role of Krt5+/p63+ basal cells in differentiation and renewal in injured and disease states present in the nasal cavity.

F-1138

NEUROG1+ PROGENITORS CAN GIVE RISE TO PRIMITIVE CELL TYPES IN THE OLFACTORY EPITHELIUM

Brian Lin[1], Julie C. Hewitt[1], James E. Schwob[2]

[1]*Sackler School of Biomedical Sciences, Tufts University, Boston, MA, United States,* [2]*Department of Anatomy and Cellular Biology, Tufts University, School of Medicine, Boston, MA, United States*

The mammalian olfactory epithelium (OE) is a powerful model for studying neural regeneration. Two broadly defined basal populations, globose basal cells (GBCs) and horizontal basal cells (HBCs), participate in regeneration after injury. HBCs are largely quiescent and appear to play the role of a reserve population and require activation through injury prior to acquiring multipotency. We have shown that the population of GBCs, on the other hand, includes cells that function as multipotent progenitors through transplantation assays wherein GBCs FACS-sorted from normal OE give rise to neurons, sustentacular cells, duct/gland cells, and other GBCs. However, GBCs are a heterogeneous population that encompasses Sox2/Pax6 (+) multipotent cells, neuron-fated Ascl1 (+) that are transit-amplifying elements, and neuron-producing Neurogenin1/NeuroD1 (+) variants. This hierarchical model of GBC fate is based on the epistatic consequences of gene knockout and temporal correlation during recovery after epithelial injury. A more stringent test of progenitor cell capacity/commitment requires isolation of the different subtypes and challenge by transplantation into MeBr-lesioned OE where multipotency is called for. Thus, to delineate the differentiative potential of different GBC variants we have bred the double transgenic mice Neurog1-eGFP::CAG-TdTomato and Sox2+/eGFP::CAG-TdTomato and used them as the source of donor cells in our transplant assay. Transplantation of FACS-purified Sox2+ GBCs confirms that they are pluripotent and form all cell types of the OE. Surprisingly, transplantation of the Neurog1+ GBC population reveals a previously unanticipated plasticity, as the Neurog1(+)-derived cells generate clones containing neurons and sustentacular cells, as well as HBCs. Post-hoc assessment of sorted Neurog1-GFP (+) cells demonstrates negligible (far less than 1%) contamination by HBCs or Sox2 (+) GBCs, confirming the potency of the Neurog1 (+) variant. The results provide an indication that the apparent hierarchy actually implies differentiative fate and not commitment and that the GBC population is remarkably plastic in its ability to shift to a more primitive multipotency. Supported by NIH grant R01 DC002167.

Embryonic Stem Cell Clinical Application

F-1141

IMMUNOGENIC PROPERTIES OF AUTOLOGOUS AND ALLOGENIC NEURAL AND CARDIOVASCULAR PROGENITORS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Victoria L. Glenn[1], John Paul Schell[1], Ronald Coleman[1], Eyitayo Fakunle[1], Candace L. Lynch[1], Bianca Mothe[2], Suzanne E. Peterson[1], Jeanne Loring[1]

[1]*The Scripps Research Institute, La Jolla, CA, United States,* [2]*CSU San Marcos, San Marcos, CA, United States*

Human pluripotent stem cells (hPSCs) are a promising cell source for regenerative medicine because they can replicate indefinitely and generate any cell type in the body. However, before these cells may be used clinically, it is of great importance to assess their antigenicity and immunogenicity and determine whether it will be necessary to immune match them to the recipient. The Major Histocompatibility Complex (MHC) enables immune cells to distinguish “self” from “non-self” and plays a critical role in graft rejection. Early reports suggested that undifferentiated hPSCs were non-immunogenic, in that they expressed little to no MHC and did not evoke an immune response even in an allogenic setting; however more recent publications have raised concerns regarding these findings. Because progenitors of differentiated cells derived in vitro are more likely than undifferentiated hPSCs to be used therapeutically, it is more clinically relevant to determine the immunogenic properties of these early derivatives. Two progenitor cell types of particular interest in regenerative medicine are neural progenitor cells and cardiovascular progenitors, which have been proposed for use in treating a wide range of neurodegenerative and cardiovascular disorders. We have generated and characterized a group of human iPSC lines with a variety of different MHC haplotypes. For each line, expression of MHC and other immune ligands was examined in both undifferentiated cells and cells differentiated into neural and cardiovascular progenitors, using a combination of flow cytometry and gene expression analysis. Consistent with previous reports, our data show expression of immunogenic antigens increases as the cells differentiate. To address the immune stimulating potential of the cell types, mixed lymphocyte reactions were carried out in both allogenic and autologous settings. Responses were measured by cytokine production and cell proliferation and analyzed using FACS. Surprisingly, our initial studies suggest that even undifferentiated hPSCs may induce proliferation in an allogenic context, al-

though it is unclear yet if this proliferation is a suppressive or cytotoxic T cell population. Though this work is ongoing, it is beginning to shed light on the antigenicity and immunogenicity of hPSCs and their derivatives.

F-1142

DERIVATION OF MART-1 SPECIFIC CYTOTOXIC T LYMPHOCYTES FROM HESC

Zoran Galic, Dimitrios Vatakis, Richard Koya, Begonya Comin-Anduix, Helen Brown, Nianxin Zhong, Hongying Chen, Antoni Ribas, Jerome Zack
Medicine, UCLA, Los Angeles, CA, United States

Abstract: Human embryonic stem cells (hESC) have the ability to form any type of blood cell, including T cells, as recently demonstrated by our group and others. Use of hESC for gene therapy purposes may be superior to using hematopoietic stem cells (HSC) as hESC can be easily genetically manipulated and expanded in vitro. Also, one can establish hESC lines with well characterized vector integration sites, which will minimize the potential for vector-mediated transformation events. We previously demonstrated that both co-cultures of hESC on murine bone marrow stromal cells and embryoid body cultures supplemented with cytokines result in differentiation towards the hematopoietic lineage. When we introduced these partially differentiated cells into human thymic implants in immunodeficient mice, they further developed into functional human T cells. Furthermore, we have documented stable expression of a reporter gene in T cells derived from hESC that were previously genetically modified by a lentiviral vector. Thus we have a model to produce genetically modified T cells from hESC. Importantly for clinical purposes, hESC-derived HSC could persist for the life of the individual, and thus have the potential to produce genetically modified T cells for many years.In our current studies, we have cloned the alpha and beta chains of a T cell receptor specific for the MART-1 melanoma-associated antigen. This HLA-A0201-restricted TCR was obtained from a patient with a powerful CD8 response to his tumor, which may have contributed to his extended survival. We introduced this TCR into the H1 hESC line using a lentiviral vector and established transgenic hESC lines which stably express the MART-1 TCR. We have cultured these transgenic cells for extended periods of time without a loss of cell viability, establishing that the expression of this TCR is not toxic to hESC. Subsequently, we used our embryoid body system to differentiate these transgenic hESC into T cells progenitors, which when introduced into haplotype-matched HLA-A0201+ human thymic implants in SCID-hu mice gave rise to MART TCR-expressing cells of T lineage. These cells are developmentally skewed toward the CD8 lineage, as expected given that the transgenic TCR is restricted to MHC class I. Upon activation, the hESC-derived MART-1 TCR expressing T cells produce interferon gamma and kill haplotype matched target cells in a dose dependent manner *in vitro*. Furthermore, our time lapse studies demonstrate that a single transgenic T cells is sufficient to kill a melanoma cell and that the whole process takes less than three hours. In the next set of experiments we will test the ability of the MART TCR transgenic T cells to recognize and kill tumor cells in vivo, in a mouse model of human malignant melanoma. Our studies have provided a proof-of-principle that hESC can be developed into melanoma-fighting cytotoxic T lymphocytes. However, these studies will also be relevant to other tumors expressing defined tumor antigens.

F-1143

A NOVEL METHODOLOGY OF IMMUNE REGULATION USING PLURIPOTENT STEM CELLS

Hiroya Kudo[1], Hajime Sasaki[1], Haruka Wada[1], Satoshi Kojo[1], Tatsuya Chikaraishi[2], Ken-ichiro Seino[1]
[1]Devision of Immunobiology, Institute for Genetic Medicine, Hokkaido University, Sapporo, Japan, [2]Devision of Immunobiology, Institute for Genetic Medicine, St.Marianna school of medicine, Sapporo, Japan

Recently, a promise of regenerative
medicine using pluripotent stem cells such as embryonic stem cells (ESCs) or
induced pluripotent stem cells (iPSCs) as donor source has been increased.
However, particularly when ES cells were used, immune-suppressive therapy
should be required because the donor-recipient combination would be allogeneic.
We successfully induced

immunosuppressive cells in a differentiation process with granulocyte-macrophage colony-stimulating factor (GM-CSF) from mouse ESCs. The immunosuppressive cells were similar to so-called regulatory macrophages in the meaning of cell surface molecule and gene expressions. They efficiently suppressed allogeneic T cell proliferative responses, at least in part, in nitric oxide dependent manner. We applied these cells to in vivo allogenic transplantation and found that they substantially prolonged ESCs-derived graft survival. These results open a new insight for development of a novel immune-regulatory strategy in the age of regenerative medicine based on the use of pluripotent stem cells.

F-1144

GENERATION OF A MOUSE MODEL FOR HUMAN ADIPOSE TRANSPLANTATION: A NOVEL STRATEGY TO STUDY ADIPOSE PHYSIOLOGY AND METABOLISM

Youn-Kyoung Lee, Fang Xia, Chad A. Cowan
Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States

Adipose plays critical roles in the regulation of energy homeostasis and acts as an integrator of various physiological pathways. Adipose dysfunction causes serious public health problem including obesity, type 2 diabetes, and cardiovascular diseases. As a critical mediator of metabolic homeostasis, adipose has gained attention and there is renewed interest in deriving relevant human cellular disease models to study metabolic disorders. However, human cell-based models are limited to understanding cell-autonomous phenotypes. On the other hand, animal model systems are useful tools to study complex metabolic disorders but they often do not faithfully phenocopy human physiology and disease. As most cases of human metabolic disorders represent whole-body phenomena arising from complex intercellular and inter-tissue interaction, to better understand adipose physiology and metabolism, we have developed a human adipose transplant model. Our model utilizes human pluripotent stem cell (hPSC)-derived adipocytes that are transplanted into lipodystrophic immune-compromised mice. This approach helps bridge the gap between the limitations inherent in both model systems and allows for the interrogation of human metabolic diseases in the whole body. We generated our human adipose transplant model by breeding fatless A-ZIP/F-1 transgenic mouse, which allows the repopulation of ectopic adipocytes to be promoted, with immune-compromised Rag2-/-; IL2γc double knockout mouse, which allows human adipocytes to be received without immune rejection. The A-ZIP/F-1 transgenic mice have been associated with numerous abnormal phenotypes including premature death, elevation of serum glucose, insulin, triglycerides, and the reduction of leptin due to the lack of white adipocytes and inactive brown adipocytes. Previously, we have developed a protocol to differentiate hPSCs into adipocytes by employing the inducible expression of key adipogenic transcriptional regulators and have demonstrated that these cells exhibit morphological and functional characteristics of mature adipocytes. To test whether transplantation of human adipose can rescue lipoatrophic and diabetic phenotypes of A-ZIP/F-1; Rag2-/-; IL2γc mice, we transplant programmed human adipocytes in subcutaneous flank or/and visceral fat depot area. Transplanted cells formed ectopic fat pads *in vivo* and maintained themselves for more than 10 weeks. Histological analysis revealed that these cells exhibited morphological characteristic of mature adipocytes and expressed adipose-specific markers such as PLIN1 by immunostaining. Expression of adipogenic genes, as assessed by qRT-PCR, was also detected in adipose transplants. Moreover, we found that mice with human adipose transplants showed improved glucose tolerance. Further, we are exploring the impact of cellular transplantation of adipocytes derived from hPSCs on whole-body glucose, insulin, and lipid metabolism. Taken together, this mouse model will be particularly useful for: 1) Dissecting out the effects of metabolic and endocrine communication between human adipose tissue and the rest of body, 2) Investigating phathophysiology of adipose disorders by transplanting adipocytes derived from hPSCs with various mutations causing metabolic diseases, and 3) Evaluating *in vivo* responses to pharmaceutical interventions for treating adipose disorders.

F-1145

EFFICIENT CRYOPRESERVATION OF HUMAN EMBRYONIC AND MESENCHYMAL STEM CELLS IN A CGMP PRODUCED, SERUM-, XENO-, AND DMSO-FREE, CHEMICALLY DEFINED CRYOPRESERVATION MEDIUM

Kristian Tryggvason[1], Jesper Ericsson[1], Camilla Karlsson[2]
[1]BioLamina, Sundbyberg, Sweden, [2]Cellectis, Göteborg, Sweden

Adult and embryonic stem cells bear great potential in regenerative medicine, drug discovery as well as for basic research. It is therefore highly important to establish efficient, user-friendly, and robust methods for bulk cryopreservation of these cells. Thus far, obtaining good survival after thawing has been problematic and in addition, earlier established slow-freezing protocols have resulted in cells with a high tendency to spontaneously differentiate.
We have developed a novel, current good manufacturing practice (cGMP) produced, chemically defined, xeno- and dimethyl sulfoxide (DMSO)-free cryopreservation medium denoted FREEZEstem for cryostorage and banking of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) but also human mesenchymal stem cells (hMSCs) and fibroblasts. These cells were bulk frozen in FREEZEstem, CryoStor CS10 (a chemically defined cryopreservation medium containing 10% DMSO), and control medium containing fetal calf serum and DMSO. Viability after thawing, toxicity of the freezing media as well as the impact of different thawing solutions was assessed. The cellular phenotype was evaluated using immunohistochemistry or flow cytometry analysis and the proliferation and differentiation potential were investigated.
Directly after thawing, the same or a higher number of hESC and iPSC colonies were detected for cells frozen in FREEZEstem compared to those frozen in CryoStor CS10. If left post-thaw in the media, all cell types tested frozen in FREEZEstem displayed a higher viability compared to cells frozen in CryoStor CS10. Cells frozen in FREEZEstem are also less sensitive to the composition of the thawing solution compared to cells frozen in CryoStor CS10. Abundant expression of stem cell markers, high proliferation rate and extensive differentiation potential was detected for cells frozen in all three media.
FREEZEstem cryopreservation medium not only has the advantage of being cGMP manufactured and DMSO-free, it is also a very user-friendly, robust freezing medium with very low toxicity thus offering an excellent, simple option for banking pluripotent stem cells but also other human cell types.

F-1146

GENERATION OF FUNCTIONAL MONOCYTE/MACROPHAGES BY GENETICAL CORRECTION OF PATIENT-SPECIFIC IPSC IN CONGENITAL PULMONARY ALVEOLAR PROTEINOSIS

Nico Lachmann[1], Christine Happle[2], Doreen Lüttge[1], Mania Ackermann[1], Adele Mucci[1], Nicolaus Schwerk[2], Sylvia Merkert[3], Axel Schambach[4], Gesine Hansen[2], Thomas Moritz[1]
[A]RG Reprogramming, [B]Department of Pediatric Pneumology, Allergology and Neonatology, [C]Department of Cardiothoracic, Transplantation and Vascular Surgery, [D]Institute of Experimental Hematology, [1]Hannover Medical School, Hannover, Germany

Introduction: Pulmonary Alveolar Proteinosis (PAP) due to a deficient GM-CSF/IL-3/IL-5 receptor (CSF2R) constitutes a severe lung disease caused by the functional insufficiency of alveolar macrophages, which require GM-CSF signalling for terminal maturation and intracellular processing of phospholipids. Standard treatment options for PAP are limited, however, recently intratracheal (i.t.) transplantation of healthy monocyte/macrophages (M/M) has been suggested as an effective therapy. Thus, we here have evaluated the suitability of patient-specific, iPSC-derived M/M for functional disease modelling and, following gene correction, as a donor source for i.t. transplants. Methods&Results: Patient-specific PAP-iPSC were generated from CD34+ bone marrow cells of a GM-CSF α-chain (CSF2RA)-deficient PAP patient utilising lentiviral, OCT4/SOX2/KLF4/c-Myc-based reprogramming. Three bona fide iPSC clones were obtained characterized by SSEA4/Tra1-60 expression, endogenous OCT3/4, SOX2, and NANOG reactivation, OCT3/4-promoter demethylation, three germlayer differentiation capacity, as well as lack of chromosomal abnormalities on fluorescence-R banding and array-CGH analysis. Hematopoietic differentiation of these

PAP-iPSC clones yielded M/Ms of typical morphology and surface phenotype (CD14+, CD11b+, CD45+). While basic inflammatory functions such as IL-6 secretion remained intact in these M/Ms, GM-CSF dependent functions such as CD11b activation, GM-CSF uptake, phagocytosis, and CSF2R-downstream signalling (STAT5 phosphorylation) were markedly impaired when compared to control M/Ms derived from H9-ESCs. This phenotype faithfully reproduced the defects observed in M/Ms derived from the peripheral blood of CSF2R-deficient PAP patients. Furthermore, when PAP-iPSCs were transduced with SIN-lentiviral vector expressing a codon-optimized CSF2RA-cDNA from a combined ubiquitous chromatin opening element/ EFS1a-promoter sequence (A2UCOE/EFS1a), stable and longterm (> 5 weeks) CSF2RA-expression was observed in pluripotent cells, while iPSC growth, pluripotency, and differentiation capacity remained unaffected. CSF2RA transgene expression was maintained during hematopoietic differentiation to the M/M state, and functional analysis of these gene corrected M/Ms demonstrated almost complete restoration of GM-CSF dependant functions (CD11b activation, GM-CSF uptake, phagocytosis, CSF2R-downstream signalling) when compared to control M/Ms derived from H9-ESCs or non-corrected PAP-iPSCs. Discussion: In summary, we here not only established iPSC-lines from CSF2RA-deficient PAP patients and established M/M differentiation of PAP-iPSCs as a functionally relevant disease model, but also introduce gene corrected M/Ms obtained by myeloid differentiation of PAP-iPSCs transduced in the pluripotent state with the CSF2RA gene as a highly promising source for cell and gene therapy in CSF2R-deficiency PAP to be delivered by the intratracheal route.

F-1147

EFFICIENT DERIVATION OF HUMAN EMBRYONIC STEM CELL LINES UNDER CHEMICALLY DEFINED AND XENO-FREE CONDITIONS, FOR SAFE STEM CELL-BASED TRANSPLANTATION THERAPIES

Liselotte Antonsson[1], Sonya Piskounova[1], Riina Lampela[1], Anders Kvanta[2], Sergey Rodin[3], Karl Tryggvason[3], Outi Hovatta[1]

[1]Division of Obstetrics and Gynecology, Department of Clinical Sciences, Intervention and Technology, Karolinska Institute and Karolinska University Hospital, Stockholm, Sweden, [2]Department of Vitreoretinal Diseases, St Eriks Eye Hospital and Karolinska Institutet, Stockholm, Sweden, [3]Division of Matrix Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden

Human
embryonic stem cells (hESC) are a possible source of therapies in regenerative medicine. The challenges have been to
generate clinically compliant cultures, animal substance free and genetically normal and safe lines. The protocols should also be efficient and reliable for large scale production, according to Good Manufacturing Practice requirements. In this study,
we have optimized a protocol for the derivation of clinical grade hESC lines. Under chemically defined, feeder-free, xeno-free conditions, using a human recombinant laminin-based matrix, we have successfully established nine hESC lines that have been characterized. Using this optimized derivation method, we obtained four new derived lines out of eight plated inner cell masses. In addition, we have obtained lines from isolated single blastomeres biopsied from day thee embryos.
The
application currently addressed by our group has been generation of retinal pigmented epithelium (RPE) from hESCs. We have developed a chemically defined, feeder-free xeno-free protocol for efficient, differentiation of these cell lines, generating RPE-like cells. The differentiated cells, forming a tight monolayer, are showing the characteristic RPE cobblestone-morphology, pigmentation, and hexagonal shape and they can be grown for several passages.

F-1148

IMMUNOLOGIC AND CHEMICAL TARGETING OF A PLURIPOTENT SPECIFIC TIGHT JUNCTION PROTEIN ERADICATE TUMORIGENIC HUMAN PLURIPOTENT STEM CELLS

Uri Ben-David, Neta Nudel, Nissim Benvenisty
Hebrew Univ Stem Cell Unit, Jerusalem, Israel

The tumorigenicity of human pluripotent stem cells (hPSCs) is a major safety concern for their application in regenerative medicine, as residual undifferentiated cells can proliferate and generate tumors. Cell surface markers can be invaluable for the selective removal of undifferentiated hPSCs from differentiated cultures. However, a single marker-based cell sorting was so far insufficient for complete elimination of undifferentiated cells and prevention of teratoma formation. A well-characterized pluripotent-specific cell surface protein may thus allow the selective targeting of undifferentiated cells.
By comparing gene expression patterns between hPSCs and various differentiated cell types, we identified the tight-junction gene Claudin-6 as a cell surface marker of hPSCs. We show that Claudin-6 is highly expressed in undifferentiated cells, at both mRNA and protein levels, but is absent from adult tissues. Claudin-6 expression is quickly down-regulated during hPSC differentiation. Knockdown of Claudin-6 cannot induce cell death or differentiation of hPSCs, revealing that it is dispensable for their survival and self-renewal. However, the unique expression of Claudin-6 on the surface of hPSCs can be exploited for their selective eradication in several ways: first, we show that undifferentiated cells can be FACS-sorted out of mixed populations using an antibody against Claudin-6; next, we demonstrate that a cytotoxin-conjugated antibody can selectively target undifferentiated cells; lastly - and most importantly - we reveal that clostridium perfringens enterotoxin (CPE), a potent toxin that binds several Claudin family members, selectively destroys undifferentiated cells in a rapid and efficient manner. In-vivo teratoma experiments confirm that CPE can completely eliminate the tumorigenic potential of hPSCs.
In summary, this work identifies Caludin-6 as a novel cell surface marker of hPSCs, demonstrates several immunologic strategies to use this protein to eliminate undifferentiated hPSCs from culture, and presents a successful chemical approach to completely ablate the tumorigenic cells and prevent teratoma formation. These results should therefore facilitate the use of hPSC-derived cells in regenerative medicine.

Hematopoietic Cells

F-1151

CYCLES OF DIETARY RESTRICTION REDUCE INSULIN/IGF-1 SIGNALING TO PROMOTE STEM CELL-BASED REGENERATION AND REVERSE IMMUNODEFICIENCY

Chia-Wei Cheng[1], Gregor B. Adams[2], Laura Perin[3], Valter D. Longo[1]
[1]Univ of Southern California, Los Angeles, CA, United States, [2]Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research, Keck School of Medicine, University of Southern California, Los Angeles, CA, United States, [3]GOFARR Laboratory for Organ Regenerative Research and Cell Therapeutics, Children's Hospital Los Angeles, Los Angeles, CA, United States

Immunodeficiency is at the center of a wide range of human diseases. Here we demonstrate that multiple cycles of dietary restriction (DR) abate immunosuppression, myeloid-bias and mortality in a mouse model for chemotherapy-induced myelosuppression. Notably, DR-refeedting cycles alone increased the number of adult hematopoietic stem/progenitor cells(HSPCs) without compromising the maintenance of long-term stem cells and lymphoid potential, indicating that this extreme dietary regimen stimulates lineage-balanced hematopoietic regeneration independently of chemotherapy. Deficiencies in IIS components, analogous to those caused by DR were sufficient to protect stem cells and promote their self-renewal. These findings link IIS to nutrient sensing signaling and establish their crucial role in regulating stem cell stress-resistance, self-renewal and regenerative function.

F-1152

HOST MACROPHAGES ARE A BARRIER TO THE ENGRAFTMENT OF EMBRYONIC STEM CELL-DERIVED HEMATOPOIETIC PROGENITORS.

Heather L. Thompson[1], Bryce T. McLelland[1], Nico van Rooijen[2], Jennifer O. Manilay[1]

[1]School of Natural Sciences, University of California, Merced, Merced, CA, United States, [2]Molecular Cell Biology, Vrije Universiteit, Amsterdam, Netherlands

Embryonic stem cell-derived hematopoietic progenitors (ES-HPs) have been proposed as an alternative bone marrow (BM) source. ES-HPs display features of adult hematopoietic progenitors, but do not achieve high levels of engraftment after transplantation, even in immunocompromised hosts. We hypothesized that host macrophages (Mϕs) are a barrier to ES-HP engraftment. To test this, we cultured and transplanted CD41+ ES-HPs into sublethally irradiated NOD.SCID.γc (NSG) mice that lack B, T and NK cells, but still possess Mϕ. Less than 5% ES-HP donor chimerism in the BM and spleen was observed in NSG recipients, in contrast to >90% chimerism in adult BM-transplanted controls. In addition, we noticed a 3.5-fold increase in spleen weight and a 1.75-fold increase in splenic host F4/80+ Mϕs in ES-HP recipients. In addition, these Mϕs were physically larger by forward scatter in ES-HP recipients compared to BM transplanted controls, suggesting that Mϕs may have phagocytosed the ES-HPs. Indeed, in vitro phagocytosis assays confirmed that allogeneic Mϕs phagocytosed ES-HPs at higher levels compared to syngeneic ES-HPs, while both syngeneic and allogeneic ES-HPs were phagocytosed at a significantly higher rate than syngeneic and allogeneic adult Lin- BM targets. In line with this, ES-HPs expressed low levels of CD47, a Mϕ inhibitory ligand, by quantitative PCR. To further investigate the role of CD47, phagocytosis assays utilizing CD47-Fc are in progress. When Mϕs were eliminated with liposome-encapsulated clodronate, a 2.17-fold increase in ES-HP engraftment in the BM and 5.14-fold increase in the spleen was observed. Taken together, our data demonstrate that cultured ES-HPs are unable to regulate host Mϕ phagocytosis and suggest that F4/80+ Mϕs are a barrier to ES-HP engraftment.

F-1153

THE DARK SIDE OF HEMATOPOIETIC STEM CELL EXPANSION; IN VITRO CULTURE ENTAILS SPECIFIC DNA-HYPERMETHYLATION

Carola I. Weidner[1], Thomas Walenda[1], Qiong Lin[2], Monika M. Wölfler[3], Bernd Denecke[4], Ivan G. Costa[4], Martin Zenke[2], **Wolfgang Wagner**[1]

[1]Stem Cell Biology and Cellular Engineering, Helmholtz-Institute for Biomedical Engineering, Aachen, Germany, [2]Cell Biology, Institute for Biomedical Engineering, Aachen, Germany, [A]Department of Obstetrics and Gynecology, [B]Interdisciplinary Centre for Clinical Research, [3]RWTH University Medical School, Aachen, Germany

Hematopoietic stem and progenitor cells (HPCs) can be maintained in vitro, but the vast majority of their progeny loses stemness during culture.
In this study, we analyzed DNA-methylation (DNAm) profiles of either freshly isolated or expanded CD34+ cells cultured with or without mesenchymal stromal cells (MSCs). DNAm profiles of the CD34- progeny versus those which remained CD34+ reflect hematopoietic differentiation such as hypermethylation of CD34 and CD133. Expansion of CD34+ cells either with or without MSCs has relatively little impact on DNAm - although proliferation is greatly increased by stromal support. Notably, all cultured HPCs - even those which remained CD34+ - acquired extensive DNA-hypermethylation within seven days in vitro, particularly in up-stream promoter regions, shore-regions of CpG islands and binding sites for PU.1, HOXA5 and RUNX1. The vast majority of these DNAm changes were not related to senescence-associated DNAm changes but they were rather located in relevant developmental genes. Furthermore, DNAm changes were associated with differential expression of hematopoietic genes and aberrant splicing of DNMT3A. Low concentrations of demethylating agents (such as zebularine or epigallocatechin-3-O-gallate) slightly increased the frequency of colony-forming unit initiating cells indicating that inhibition of DNAm may support expansion of progenitor cells. However, such demethylating agents do not specifically counteract the DNAm changes which are induced by in vitro culture.

Taken together, our results demonstrate that culture expanded HPCs - even those which maintain a primitive immunophenotype - acquire significant DNAm changes. These epigenetic modifications reflect the inefficient self-renewal under in culture conditions even with stromal support. Control of epigenetic modifications during culture expansion may prevent loss of stemmness.

F-1154

CELL MEMBRANE POLARITY OF FETAL LIVER HEMATOPOIETIC STEM AND PROGENITOR CELLS IN A MURINE MODEL OF FANCONI ANEMIA

Ashley N. Kamimae-Lanning, Natalya A. Goloviznina, Peter Kurre
Pediatrics, Oregon Health & Science University, Portland, OR, United States

Fanconi anemia (FA), a bone marrow failure and cancer susceptibility syndrome, results from biallelic mutations in one of 15 known genes cooperating in a DNA repair pathway. FA-associated bone marrow failure is thought to reflect postnatal exhaustion of hematopoietic stem & progenitor cells (HSPC). However, clinical & experimental evidence suggests impaired prenatal hematopoietic function. We previously reported that *Fancc*$^{-/-}$ day 14.5 fetal mice have reductions in liver size, compromised serial repopulating capacity, and some perinatal lethality. In addition, we described a relative decrease in quiescence and an increase in G_1 phase of the cell cycle in HSPC-enriched *Fancc*$^{-/-}$ fetal liver cells. We also performed a candidate screen of HSC-relevant transcripts that revealed further unique differences between *Fancc*$^{-/-}$ and WT fetal liver cells. Intriguingly, several dysregulated transcripts including *p21*, an activator of p53, as well as *Robo4* are involved in regulation of cell polarity and migration. Niche-to-niche migration of HSPCs is not only critical during the developmental transition from fetal liver hematopoiesis to bone marrow, but also for steady-state organ function, during transplantation, and in the postnatal pathology of FA HSPCs. Transwell chemotaxis assays, performed to gain functional insight, revealed that *Fancc*$^{-/-}$ fetal liver cells are defective in their ability to respond to both stromal-derived factor-1α (SDF-1α) and stem cell factor. We also showed that overall cell surface expression of the key homing receptor for SDF-1α, Cxcr4, was not significantly reduced in *Fancc*$^{-/-}$ fetal liver cells by flow cytometry. We next tested whether *Fancc*$^{-/-}$ exhibits dysfunctional polarization to account for *in vitro* deficits in migration, and our data confirm that the membrane polarization of Cxcr4 occurred at a reduced frequency on AA4.1^{+} Sca-1^{+} Lin$^{low/-}$ *Fancc*$^{-/-}$ fetal liver HSPCs than in WT. This observation is consistent with studies by others on the association of polarized CXCR4 membrane domains with engraftment and HSPC quiescence. On the other hand, polarization of Numb, a cell fate-determining inhibitor of Notch that segregates with progenitors after asymmetric HSC division, was increased in *Fancc*$^{-/-}$ fetal liver HSPCs. Interestingly, Numb can also interact with p53 pathways. We propose that developmental deficits in symmetric division compromise *Fancc*$^{-/-}$ fetal liver HSC pool expansion during development. Our systematic study of fetal liver hematopoiesis in FA indicates broad qualitative and quantitative developmental defects that limit seeding of the fetal bone marrow. Studies to gain mechanistic insight into migration and divisional symmetry in *Fancc*$^{-/-}$ fetal liver HSPCs are currently underway. Results contrast with a conventional model of postnatal stem cell attrition and suggest a novel FA HSPC phenotype of altered polarity, coincident with known qualitative and quantitative deficits.

F-1155

FEV REQUIRED FOR MAINTENANCE OF FETAL HSC IS RE-ACTIVATED IN LSC

Tianhui Liu[1], Fang Zhang[1], GuoQiang Chen[1], Feng Liu[2], **Dengli Hong**[1]
[1]Shanghai Jiao-Tong University School of Medicine, Shanghai, China, [2]State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

Fev, also known as pet1 in mammals, is a recently identified ETS transcription factor belonging to the same subgroup as fli1 and erg. Our previous studies showed that fev is essential for homogenetic endothelium-based hematopoietic stem cell (HSC) development. It remains unknown however whether fev plays any role in maintenance of

both fetal and adult HSC and is altered in leukemic stem cells (LSC). We have addressed these issues in the human context of normal and leukemic hematopoiesis. Fev is expressed in hematopoietic cells of fetal liver and neonatal cord blood but not in that of post-natal bone marrow indicating that fev is a specific regulator of fetal hematopoiesis. Fev-knockdown in primitive hematopoietic cells of cord blood led to a marked reduction of primitive colony-forming cells (CFC), long term culture-initiating cells (LTC-IC), and SCID-repopulating cells (SRC) in functional assays in vitro and in vivo, indicating fev is crucial for maintenance of fetal HSC. Assays of CFC-replating and SRC-retransplantation showed that fev is a regulator of self-renewal in fetal HSC. More interestingly, fev is detected in leukemic cells of some cell lines and patients, which is associated with complex karyotype and clinical high-risk. Moreover, knockdown of fev in the leukemic cells markedly compromised their capacity of leukemic reconstitution in xenograft recipients, indicating that fev is reactivated and functions in LSC. Therefore, we have identified and characterized fev a candidate to distinguish LSC from normal HSC.

F-1156

HEMATOPOIETIC STEM CELL DERIVED CARCINOMA ASSOCIATED FIBROBLASTS PROMOTE TUMOR PROGRESSION

Lindsay T. McDonald, Dayvia A. Laws, Ryan R. Kelly, Victoria J. Findlay, Amanda C. LaRue
Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC, United States

Cells and paracrine factors of the tumor microenvironment play a central role in tumor angiogenesis, invasion, migration and proliferation, making the tumor microenvironment an exciting therapeutic target. Among the most prominent cell types in the tumor stroma are fibroblasts, termed carcinoma-associated fibroblasts (CAFs). We have developed a unique transplantation model in which the bone marrow of lethally irradiated recipient mice is reconstituted by a clonal population of cells derived from a single $EGFP^+$ hematopoietic stem cell (HSC). Using this model, we have identified a novel population of CAFs and CAF precursors (termed circulating fibroblast precursors, CFPs) that are of HSC origin. We have previously shown that HSC-derived CFPs are derived from the monocyte lineage and that these cells preferentially migrate and differentiate into mature CAFs in response to tumor. Inhibition of CFP recruitment using a neutralizing antibody to monocyte chemoattractant protein-1 (MCP-1) resulted in decreased primary tumor size, suggesting a critical role of these CAF precursors in tumor progression. While these single cell transplantation studies have identified a novel origin of CAFs and CAF precursors, the objective of this study is to elucidate the mechanisms by which these cells promote tumorigenesis. Using our single cell transplantation methodology in conjunction with two murine models of solid tumor, Lewis lung carcinoma (LLC-1) and melanoma (B16), we examined the functional role for HSC-derived CAFs and CFPs in both primary and metastatic tumor. Our *in vivo* studies demonstrate that co-injection of HSC-derived CAFs with tumor cells significantly enhanced tumor growth. Molecular profiling and *in vitro* analyses revealed a role for HSC-derived CAFs in promoting tumor vascularization via production of pro-angiogenic cytokines and matrix metalloproteinases. Similarly, we found that HSC-derived CFPs from tumor-bearing mice were able to significantly increase tumor cell migration and invasion, suggesting a role for these cells in tumor cell extravasation/intravasation. We also found that CFPs from tumor-bearing mice were able to promote tumor cell proliferation *in vitro*. This finding in combination with our *in vivo* data demonstrating the presence of HSC-derived CFPs in the lung during metastatic disease, suggests that in addition to a role in primary tumor, these cells may play a role in tumor cell colonization of the metastatic site. Collectively, our studies suggest that HSC-derived CAFs and CFPs play a critical role in tumor progression at both the primary and metastatic tumor site, thus representing potential multi-phase targets for novel therapies directed at fibroblasts in the tumor microenvironment.

F-1157

THE UNIQUE ROLE OF GATA-3 IN HEMATOPOIETIC STEM CELL MAINTENANCE

Chia-Jui Ku, Tomonori Hosoya, Ivan Maillard, James D. Engel
Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, United States

Hematopoietic stem cells (HSC) are critical progenitors required for the life-long generation of the multiple hematopoietic lineages. Maintaining the balance between quiescent and cycling of HSC is essential both for continuous hematopoiesis as well as retaining the capacity for long-term repopulation.
Transcription factor GATA-3 is known to be a critical regulator of T cell development. Although it has been well documented that GATA-3 is expressed in HSC, it was only recently that a functional role for GATA-3 in HSC was reported. Our continuing studies have focused on unique aspects of GATA-3 regulatory function(s) in HSC.
We have recently determined that GATA-3 is expressed in a highly purified HSC population (LSK $CD150^{+}$ $CD48^{-}$ $CD34^{-}$) in the bone marrow and is required for adult HSC maintenance through it's regulation of cell cycle entry. When conditional *Gata3* mutant ablation was implemented using Mx1Cre, the number of HSC was reduced to 49% of controls in the bone marrow of 9-12 week old adult mice. Cell cycle/quiescence analyses conducted by examining bone marrow cells for incorporation of 5-bromodeoxyuridine (BrdU) or by acquisition of Ki67 showed that the percentage of HSC that exited G0 (quiescence) and entered the cell cycle diminished in *Gata3* mutant cells, thus demonstrating that GATA-3 is intimately involved in recruiting HSC out of a quiescent state. G0 HSC are known to be stimulated into cycle after injury (e.g. treatment with 5-fluorouracil; 5-FU). Proper transition from quiescent to cycling status is critical to recovery from 5-FU stress. When challenged to recover after 5-FU administration, LSK cells in *Gata3* mutant animals exhibited no enhancement of cycling but displayed impaired expansion. The reduced reconstitution potential of *Gata3* mutant ($Gata3^{flox/flox}$:Tg^{Mx1Cre}) HSC was confirmed in adoptive transfer experiments: after 16 weeks of reconstitution of lethally irradiated recipients, *Gata3* mutant HSC showed significantly reduced expansion in comparison to *Gata3* control HSC, defining a cell intrinsic role for GATA-3 in HSC homeostasis. GATA-2 is another critical regulator of HSC/progenitor function. GATA-2 and GATA-3 have been shown to be at least partially compensatory in other biological contexts, and therefore it was possible that GATA-2 might compensate for deficient GATA-3 abundance (GATA-2 is approximately 25-fold more abundant than GATA-3 mRNA) in HSC. To test for possible compensatory activity, we analyzed the effect of conditional *Gata3* loss in heterozygous and homozygous *Gata2* mutant backgrounds. GATA-2 loss results in a haploinsufficient deficiency by half in HSC number in adult animals. In a *Gata2* heterozygous or homozygous mutant background, conditional loss of *Gata3* results in a (additional) 55% loss of HSC, indicating that GATA-2 cannot compensate for loss of GATA-3 in HSC. To further investigate the function of GATA-3 in regulating HSC cell cycle, we also monitored the viability of multiple genotypes mice for long periods after 5-FU administration. These experiments showed that *Gata3* mutant mice exhibited higher mortality when compared to control mice. The same experiment performed in a *Gata2* heterozygous background demonstrated that GATA-2 abundance does not affect the age-sensitive lethality of *Gata3* conditional loss. These results suggest a unique, GATA-2 independent role for GATA-3 in HSC activity.

F-1158

AUTOLOGOUS BONE MARROW CELLS TRANSPLANTATION ASSOCIATED TO BYPASS CORONAY GRAFTS IN PATIENTS WITH LOW EJECTION FRACTION(20-30%)AND CHRONIC HEART FAILURE

Alfonso Rivas-Plata[1], Marcos Pariona[2], Ausberto Chunga[3], Carlos Quispe[1]
[A]Thoracic and Cardiovascular Surgery, [B]Cardiology, [C]Clinical Pathology, [1]Hospital Rebagliati, Lima, Peru

From November 2004 till July 2012,66 patients(89%male and 11%female) were operated with CABG and implantation of autologous bone marrow stem cells.The preop diagnosis in all patients were heart failure with multiple myocardial infarctions,with a ejection fraction between 20 and 30%.Pre and post op. studies included a pre op coronary angiogram ecocardiogram(once a month in the first year and every three months up to five years)myocardial perfusion Thallium study(every three monts up to the first two years,and every 6 months up to five years).Excluding criteria were a 50% or more necrosis of the left ventricule,and a end systolic left ventricule volume index of 100ml or more.In hospital mortality was 6% because heart failure and late mortality of 4.5% because non cardiac related events.The mean number of injected intramyocardial mononuclear cells was 400 millions including 1-3% of CD34 stem cells
There was an mean increase of E.F.from 27% to 41% at one year and 39% at 5 years follow up.Mean follow up was 4.5 years.Improvement of regional contractility index of the left ventricule,funtional class and quality of life were

observed.In the last 20 patients and extra 6 ml. source of stem cells were added into a collagen patch sutured on top of the myocardial infartion area.The results in these sub group show a higher better improvement of the EF in relation to the original group
In conclusion aplication of autologous bone marrow stem cells showed improvement of the left ventricular funtion and will probably delay or avoid the need of heart transplantation.

F-1161

PROMOTION OF HELPER T CELL DIFFERENTIATION FROM MOUSE INDUCED PLURIPOTENT STEM CELLS BY THPOK TRANSDUCTION

Tomoko YAMAGUCHI[1], Misae NISHIJIMA[1], Yasutaka YAMANAKA[1], Katsuhisa TASHIRO[1], Hiroyuki MIZUGUCHI[2], Kenji KAWABATA[1]

[1]Laboratory of Stem Cell Regulation, National Institute of Biomedical Innovation, Osaka, Japan, [2]Laboratory of Biochemistry and Molecular Biology, Graduate School of Pharmaceutical Sciences, Osaka University., Osaka, Japan

Because of their pluripotency and self-renewal, ES cells and iPS cells are potential cell sources for regenerative medicine and other clinical applications, such as cell therapies, drug screening, toxicology, and investigation of disease mechanisms. One of the possible applications of iPS cells is to use them as a cell source for producing lymphocytes for cell-based therapy against cancer and infectious diseases. In this study, we report that mouse iPS cells that were co-cultured with OP9-DLL1 cells could differentiate into CD4- and CD8-single positive (SP) cells. When co-cultured with OP9/DLL1 cells, iPS cells efficiently generated CD4/CD8-double positive (DP) cells and CD8 SP cells. To determine whether the TCRs expressed on these T cells were indeed functional, we stimulated the cells in the plate-bound anti-CD3 antibody. Some populations of the iPS cell-derived T cells produced IFN-γ in response to the TCR stimulation. On the other hand, iPS cells were relatively resistant to CD4-SP cell lineage differentiation. To investigate whether forced expression of transcription factors could promote helper T cell differentiation, iPS cell-derived CD4/CD8-double negative (DN) cells were transduced with ThPOK-expressing lentivirus vectors. The transduction of ThPOK resulted in a significant increase in the number of CD4-SP cells compared with non-transduced cells. In turn, the number of CD8-SP cells was decreased in ThPOK transduced cells. These results indicate that ThPOK expression is sufficient to augment the CD4 helper T cell differentiation of iPS cells, and our method would be useful for clinical applications.

F-1162

ENDOTHELIAL AND MESENCHYMAL-LINEAGE CELLS DERIVED FROM PLURIPOTENT STEM CELLS HAVE DIFFERENT CHARACTERS IN SUPPORTING HEMATOPOIETIC PROGENITORS

Akira NIWA, Magumu K. Saito, Tatsutoshi Nakahata
Kyoto University, Kyoto, Japan

Efficient induction and expansion of hematopoietic stem/progenitor cells have been of great interest among medical scientific fields. For that purpose, it is certainly important to understand the hematopoietic homeostasis in which blood cells emerge, proliferate and are maintained throughout life.
During embryogenesis, hematopoietic cells first emerge from and are supported by endothelial cells located in the aorta-gonad-mesonephros (AGM) regions, and finally moved to bone marrow via fetal liver. Although the detail mechanisms are not yet clear, stroma cells in those microenvironments are thought to support the survival and commitment of hematopoietic progenitors. In fact, the functional impairments in bone marrow stroma have been reported to cause some hematological defects in vivo.
In hematology, ESC/iPSCs can become not only a tool to investigate normal and impaired hematological system but also a feasible cell source to expand hematopoietic stem cells for transplantation therapy. Despite the recent establishment of several methods to generate human ESC/iPSC-derived blood and other somatic cells, however, ESC/iPSC-derived stromal cells for sufficiently supporting hematopoietic lineage have never been reported so far.

Here we report the human ESC/iPSC-derived stroma cells capable of efficiently supporting hematopoietic cell survival and lineage commitment in vitro. We manually lifted non-hematopoietic cells from our previously reported human ESC/iPSC-derived hematopoietic culture system, expanded them in serum-free culture condition and analysed the blood cell supporting potential. Intriguingly, stromal cells with endothelial characters and mesenchymal characters have different potential to support cord blood-derived hematopoietic stem/progenitor cells. Endothelial stroma has stronger potentials to expand immature progenitor cells, while mesenchymal stroma can expand later stage progenitors. By use of this stroma, we can investigate the detail mechanisms of supporting human hematopoietic cells in vivo.

F-1163

CNR2 SIGNALING MODULATES HEMATOPOIETIC STEM CELL DEVELOPMENT VIA PGE2 DEPENDENT AND INDEPENDENT MECHANISMS

Virginie E. Esain[1], Gregory Frechette[1], Leah Liu[2], Lea Sheward[1], Kelli J. Carroll[1], Wolfram Goessling[2], Trista E. North[1]

[1]Pathology, Beth Israel Deaconess Medical Center, Boston, MA, United States, [2]Genetics, Brigham and Women's Hospital, Boston, MA, United States

Cannabinoid-related compounds can modulate the survival and the migratory behavior of healthy and leukemic hematopoietic cells. In an irradiation recovery assay, the absence of the cannabinoid-receptor 2 (CNR2) is detrimental to the survival of hematopoietic stem and progenitor cells (HSPC) whereas its stimulation enhances HSPC recovery. However, the mechanism of action of cannabinoid receptor signaling in HSC formation remains elusive. In vertebrates, definitive hematopoietic stem cells (HSCs) arise in the embryo from hemogenic endothelium within the aorta-gonad mesonephros (AGM) region; in the zebrafish, runx1;cmyb-dual positive HSCs begin to appear in the AGM by 30 hours post fertilization (hpf). By 36hpf, these HSCs start to migrate from the AGM to the caudal hematopoietic tissue (CHT), the fish equivalent of the fetal liver for HSC maturation. Using chemical approaches to target cannabinoid receptor (CNR1 and CNR2) signaling, we show that CNR2- but not CNR1-stimulation increases HSC formation in the AGM and in the CHT regions; likewise, morpholino knockdown revealed CNR2, but not CNR1 is required for normal HSC development. As the related eicosanoid species Prostaglandin E2 (PGE2) can modulate embryonic HSC specification and adult homeostasis, we next analyzed the combined effect of CNR2 and PGE2 stimulation on HSC regulation. Together, PGE2 and CNR2-agonists increase HSC number in the AGM to a higher level than each of these compounds alone. Further, CNR2-stimulation is able to rescue chemical and genetic knockdown of PGE2 production, except when the expression of the PGE2 receptors (EP) and cox2 is reduced. Using a chemical approach, we show that COX-2 activity, downstream of CNR2-stimulation, is required to enhance HSC development only in the AGM but not in the CHT. To further delineate the mechanism of action of the PGE2 and CNR2 pathways, we analyzed the cell proliferation in the AGM and in the CHT regions. Both pathways can increase the number of phospho-histone H3-positive cells in the AGM and in CHT; however, the effect of CNR2 stimulation in the AGM, but not the CHT, requires COX-2 activity, suggesting it may be mediated in part by upregulation of PGE2 production. Using RT-qPCR and a PGE2 biochemical assay, we show that CNR2 stimulation can increase PGE2 production during hematopoietic niche specification between 12 and 24hpf in the developing embryo, a previously uncharacterized mechanism of action of CNR2 signaling in hematopoietic regulation. Interestingly, in a zebrafish model of marrow recovery after injury, we show that PGE2 and CNR2 signaling perform similar interactions to regulate HSPC homeostasis. Collectively, these data provide a nuanced view of the function of the cannabinoid signaling in HSC formation and homeostasis, that incorporates its intersection with PGE2-mediated regulation. Due to its differential mechanisms of action in vivo, the modulation of the cannabinoid signaling provides new therapeutics strategies for HSC regulation.

F-1164

IDENTIFICATION OF THE EARLIEST CLONALLY MULTIPOTENT HEMATOPOIETIC STEM AND PROGENITOR CELLS IN MOUSE EMBRYONIC DEVELOPMENT

Matthew A. Inlay[1], Thomas Serwold[2], Adriane Mosley[1], Irving L. Weissman[1]
[1]ISCBRM, Stanford, Stanford, CA, United States, [2]Joslin Diabetes Center, Boston, MA, United States

Hematopoietic development in the embryo proceeds in a series of waves, with primitive erythoid-biased waves succeeded by definitive waves within which the properties of HSCs (multilineage potential, self-renewal, engraftability) gradually arise, until fully functional HSCs emerge. The identity of cells that give rise to each wave of hematopoiesis and the tissues they emerge from remains unclear, hampering efforts to dissect their developmental relationship. While the properties of self-renewal and engraftability have been examined in the embryo, multilineage potential has been less characterized. In order to identify when clonal multilineage potential arises in embryonic development, and identify the populations that possess it, we developed our own novel single cell multipotency assay, and used it to identify multipotent cells during embryonic development. We found a population, representing a rare fraction of ckit+Sca+ cells, that is present in the YS and AGM at e9.5, and the FL at e11.5, that contains multilineage potential at the single cell level. From e9.5 to e11.5, the YS contained more multipotent cells than either the AGM or FL. However, at e12.5, the FL possessed the most multipotent cells. By surface phenotype, this population was identical in all tissues examined, at all timepoints, and appears similar to pre-HSCs, which are known to self-renew, but cannot engraft unless transplanted into neonatal mice or pre-cultured on stroma. Our data suggest that YS cells can contribute to definitive hematopoiesis, and that the properties of multipotency and self-renewal appear simultaneously in both the YS and AGM, prior to the ability to engraft.

F-1165

IMPAIRED ERYTHROPOIESIS OF GFI-1 NULL HEMATOPOIETIC PROGENITOR CELLS IS RESCUED BY REDUCING ID2 LEVELS

WONIL KIM, Kimberly D. Klarmann, Jonathan R. Keller
Mouse Cancer Genetics Program, SAIC-Frederick/FNLCR, Frederick, MD, United States

The survival, self-renewal and differentiation of hematopoietic stem and progenitor cells (HSPC) are tightly regulated by extrinsic signals from the microenvironment, and intrinsically by transcription factors and transcriptional networks. Previously, we determined that inhibitor of DNA binding/differentiation 2 (Id2) is a physiological regulator of B cell development, and that growth factor independence-1 (Gfi-1) is linked to this mechanism by negatively regulating the expression of *Id2*. Gfi-1 is required for the development of multiple cell lineages including HSPC and ultimately differentiated blood cells, however, the mechanisms by which Gfi-1 mediates these effects are currently unknown. Since *Id2* expression is elevated in HSPC and Id2 can promote cell proliferation, we hypothesized that lowering *Id2* expression could rescue the HSPC defects observed in *Gfi-1* knockout mice. By transplanting *Gfi-1* knockout mouse bone marrow into lethally irradiated recipient mice, we observed that short-term reconstituting activity is rescued by heterozygosity at the *Id2* locus, while the long-term reconstitution defect of HSC was not. Furthermore, we identified that reduced levels of *Id2* expression restore erythroid cell development by rescuing short-term hematopoietic stem cell, common myeloid progenitor and megakaryocyte erythroid progenitors in the *Gfi-1* null mice. In addition, burst forming unit-erythroid (BFU-E) colony assay and Benzidine staining showed that hemoglobinized erythroid cell development was restored in *Gfi-1* knockout bone marrow and spleen by lowering Id2 levels. Since abnormal expansion of pro-erythroblasts during *Gfi-1* null erythroid development was rescued by reducing *Id2* expression, we hypothesized that erythroid development was blocked at an early stage due to ectopic expression of Id2 in *Gfi-1* knockout mice. Using *Id2* promoter-driven YFP reporter mice, we found that *Id2* expression is decreased as pro-erythroblasts mature confirming that *Id2* expression is repressed in later stages of differentiation. The dramatic changes of *Id2* expression during erythroid development support our findings that the overexpression of Id2 which occurs in the absence of Gfi-1-mediated transcriptional repression causes impaired erythropoiesis at the pro-erythroblast stage. To identify the molecular mechanisms that could account for how reduced Id2 levels rescue erythropoiesis in *Gfi-1* knockout mice, we examined expression of genes and proteins associated with erythroid proliferation and differentiation. Using microarray, qRT-PCR, and western blotting, we discovered that reduction of *Id2* expression in *Gfi-1* knockout mice results in increased expression of *Gata1*, *EKlf*, *EpoR1*, and *Tfrc* genes, which are essential for erythroid differentiation. However, the expression levels of cell cycle regulators

were not altered by lowering *Id2* expression in *Gfi-1* null mice. These data may suggest a novel molecular mechanism in which Gfi-1 modulates erythroid differentiation by repressing the expression of Id2, which reduces Id2 protein binding to and inhibition of E2A. We predict this would result in enhanced E2A/SCL protein complex formation and activate the expression of Gata1. Since patients with *GFI-1* mutations have moderate anemia in addition to severe congenital neutropenia (SCN), we propose that a treatment which down modulates *ID2* expression may promote erythropoiesis in the SCN patients and improve their quality of life.

F-1166

NEW INSIGHTS INTO HEMATOPOIETIC STEM AND PROGENITOR CELL LINEAGE CONTRIBUTIONS AND KINETICS VIA CLONAL BARCODING IN A NON-HUMAN PRIMATE MODEL

Brian Li[1], Chuanfeng Wu[1], Rong Lu[2], Alexander Jares[1], Robert E. Donahue[1], Irving Weissman[2], Cynthia E. Dunbar[1]
[1]Hematology Branch, NHLBI, Bethesda, MD, United States, [2]Stem Cell Institute, Stanford University School of Medicine, Palo Alto, CA, United States

Quantitative tracking of clonal output from individual hematopoietic stem and progenitor cells (HSPCs) will be valuable for understanding normal and abnormal hematopoiesis. Limit dilution transplantation is non-physiologic and not feasible in large animals or humans, and viral insertion site tracking via techniques such as LAM-PCR are not quantitative. For the first time in a large animal or human we have applied a powerful barcoding approach to quantitatively analyze output from individual HSPCs. High diversity libraries consisting of 35bp random DNA barcodes were delivered to target cells via lentiviral vectors, under conditions resulting in a single barcode per cell. Low cycle PCR followed by Illumina sequencing was performed to identify and quantify individual barcode frequency, and to compare clonal contributions between time points and lineages. The data was analyzed using a custom computer algorithm.
We established the fraction of barcode reads retrieved from sequencing accurately represented clonal abundance in a polyclonal setting, via analyzing mixtures of 10 individual barcoded K562 clones (r=0.82). Two macaques received transplants of barcoded autologous CD34+ HPSCs following total body irradiation. Peripheral blood from 1, 2, 3, 4.5 and 6.5 months post-transplant was FACS sorted into highly purified granulocytes, monocytes, T, B, and NK cells. Bone marrow CD34+ and mature cells were collected from left and right pelvic sites at 5 months. The fraction contribution of individual barcoded clones within each sample was assessed. Duplicate samples were analyzed to confirm the reproducibility of quantification of clonal output via this method (r=0.99).
To date we have detected and tracked 988 individual barcoded clones in the first animal and several hundred in the second. Clones contributing at 1 month were primarily uni-lineage, and disappeared at later time points, formally demonstrating that polyclonal, short-lived and lineage-restricted progenitors are responsible for initial hematopoietic recovery. Clones contributing by 2m in general continued to contribute at 6.5m, at relatively stable levels when tracking individual clones. Beginning at 2m, there were significantly correlated (r=0.68) and increasing (r=.0.91) shared clonal contributions to granulocytes and monocytes, formally documenting the activity of a common granulocyte-monocyte progenitor in vivo. T and B clonal contributions become weakly correlated starting at 3m (r=0.36) and increased by 6.5m (r=0.63). There was also significant overlap between clones contributing to B or T cells and granulocytes (r=0.76 and 0.71 respectively) by 6.5m, suggesting contributions from multi-lineage HSPCs. Surprisingly, clones contributing to NK cells did not correlate with any other lineage, even by 6.5m, with clones detected only in NK cells. A comparison of clones in CD34+ cells obtained from the left versus right pelvic marrow at 5m were almost completely non-overlapping, suggesting that HSPCs reconstitute local niches with little migration, at least up to 5 months post-transplant. Clonal composition of CD34- cells and granulocytes from the same locations were correlated with the CD34+ cells from the same sites.
Our quantitative characterization of hematopoiesis in the macaque provides novel insights into many aspects of stem and progenitor cell behavior, including frequency, kinetics, localization, and lineage output, studied in a physiologic and clinically-relevant model.

F-1167

OMICS LANDSCAPE OF HEMATOPOIETIC STEM CELLS AND MULTIPOTENT PROGENITORS

Nina Cabezas-Wallscheid[1], Daniel Klimmeck[1], Jenny Hansson[2], Lisa Dohrn[1], Alejandro Reyes[2], Wolfgang Huber[2], Jeroen Krijgsveld[1], Andreas Trumpp[1]

[1]Stem Cells and Cancer, German Cancer Research Center, Heidelberg, Germany, [2]Genome Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany

In the hematopoietic system, hematopoietic stem cells (HSC) harbor the highest self-renewal activity and generate a series of multipotent progenitors (MPP) that differentiate into lineage-committed progenitors and subsequently mature cells. However, despite intense research efforts over the last decades the molecular basis of essential HSC features such as self-renewal and quiescence remains poorly understood. To explore these comprehensively and gain further insights into regulation of gene expression in early hematopoiesis, we performed an extensive global OMICS analysis combining the latest generation of quantitative proteome and transcriptome (RNASeq) analyses. To do this, we FACS-sorted mouse HSC (Lin^{neg} Sca-1+ cKit+ ,LSK, CD34- Flt3- CD150+ CD48-), MPP1 (LSK CD34+ Flt3- CD150+ CD48-), MPP2 (LSK CD34+ Flt3- CD150+ CD48+), MPP3 (LSK CD34+ Flt3- CD150- CD48+) and MPP4 (LSK CD34+ Flt3+ CD150+ CD48+) populations as previously described in our laboratory (Wilson et al.). By employing stable isotope dimethyl labeling and high-resolution tandem mass spectrometry in biological triplicates, more than 7,000 proteins were identified including over 400 transcription factors (TF) and 180 plasma membrane receptors. Expression profiling highlights energy metabolism, immune response, cell cycle and DNA repair to be modulated during early differentiation and lineage comittment. To our knowledge, these data represent the first global protein signature of HSCs defined at this level. Furthermore, using in-depth RNASeq we achieved more than 11-fold coverage of the genome enabling the robust quantification of over 22,000 genes. We describe specific expression clusters of cell adhesion molecules and TFs characteristic for each of the respective populations. Since little is known about the expression of regulatory long non-coding RNAs (lncRNA) in HSCs or MPPs, we filtered for polyA-lncRNA. This revealed a comprehensive landscape of these lncRNA including more than 50 being differentially expressed. Finally, using these OMICS data sets we explored the hierarchy between HSC and MPP populations by performing rigorous statistics and bioinformatic cluster analyses. Principle component analysis of the transcriptome data depicts HSC and MPP1 clustering together. Notably, MPP2 are found closer to HSC/MPP1 than MPP3 and MPP4. Since the differentiation potential of MPP2, 3 and 4 towards myelo-, lympho-, or erythroid lineage has not been experimentally addressed we complemented our OMICS approach with functional transplant experiments. In summary, the transcriptome and proteome signatures for stemness and multipotency defined in this study represent a novel unique resource for the scientific community and will significantly extend the current understanding of HSC-progenitor biology at the global level.

F-1168

SUCCESSFUL ISOLATION OF HEMATOPOIETIC STEM CELLS FROM CRYOPRESERVED PLACENTAL TISSUE USING COMPLETELY CLOSED PERFUSION SYSTEM

HIROKAZU IKEDA, Daisuke Toyama, Keiichi Isoyama
Pediatrics, Showa University Fujigaoka Hospital, Yokohama, Kanagawa, Japan

Background
Recently, the placenta has been considered as a valuable source of hematopoietic stem cells (HSCs) for cell therapy. We have previously reported the presence of a significant amount of viable HSCs in human placenta, which can be recovered from fresh human term placenta using a perfusion system. However, a significant disadvantage of this system is the risk of maternal cell contamination because perfusion systems are not completely closed. Therefore, we developed a completely closed-circuit placental perfusion system to prevent maternal cell contamination and successfully isolated HSCs from cryopreserved placental tissue. In this study, we have described this breakthrough isolation technique and evaluated the effects of cryopreservation on HSCs.
Material and Methods

A total of 26 normal placentas were obtained from consenting mothers undergoing cesarean delivery of full-term infants. After delivery, the placentas were quickly cleaned with sterile saline solution, covered with sterile gauze, and cryopreserved in a double Ziploc bag at −80°C for 30 days. Following storage, the placentas were thawed and then perfused with 500 ml heparinized medium for 60 min using our novel, completely closed perfusion system. The HSCs were recovered from the perfusate by centrifugation and immediately thereafter the effects of cryopreservation were evaluated.

Results

Using our novel, completely closed perfusion system, we obtained an average of $4.9 \pm 9.7 \times 10^7$ nucleated cells and a total of live CD34+/CD45 cells of $0.54 \pm 0.22 \times 10^6$ from cryopreserved placental tissue. Compared with prefreeze, cryopreservation did not significantly decrease 7-aminoactinomycin D viability, the proportion of CD34+ cells, or the CD34+/CD38− cells, which is an indicator of primitive HSCs. Fluorescence in situ hybridization analysis detected no maternally derived XX chromosomes. In addition, bacterial and fungal contamination was not observed.

Conclusion

Our group successfully isolated HSCs from 30-day-old cryopreserved placental tissue using a completely closed-circuit placental perfusion system and avoided contamination by maternally-derived cells, bacteria, and fungi. Thus, we believe that this novel technique will facilitate a new source of HSCs.

F-1171

STABLE ENGRAFTMENT AND EFFECTIVE MGMT-MEDIATED IN VIVO SELECTION OF HEMATOPOIETIC PROGENITORS DERIVED FROM NONHUMAN PRIMATE INDUCED PLURIPOTENT STEM CELLS

Jennifer L. Gori[1], Jason M. Butler[2], Brian C. Beard[1], Devikha Chandrasekaran[1], Jennifer E. Adair[1], Michael Ginsberg[2], Daniel J. Nolan[2], Shahin Rafii[2], Hans-Peter Kiem[1]

[1]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, United States, [2]Genetic Medicine, Weill Cornell Medical College, New York, NY, United States

Clinical translation of pluripotent stem cell (PSC) technology for the production and transplantation of hematopoietic progenitor cells is impeded by the low engraftment potential of human PSC-derived $CD34^+$ cells in immunodeficient mice. This engraftment block suggests that additional angiocrine and hematopoietic cues must be provided during ex vivo development to generate cells with engraftment potential. Additionally, induced (i)PSCs could be genetically modified to provide a selective mechanism to increase the level of engrafted cells after transplantation. To advance clinical translation of iPSC therapeutics, we hypothesized that modifying iPSCs to express the transgene P140K, the O^6-benzylguanine(O6BG)-resistant variant of methylguanine methyltransferase (MGMT), would support in vivo selection of engrafted iPSCs. We further hypothesized that expansion of iPSC-$CD34^+$ cells in co-culture with Akt-activated human endothelial cells, afforded by transduction with the E4ORF1 gene ($E4^+$ECs), would provide the necessary developmental signals to augment hematopoietic cell development and engraftment in immunodeficient mice. We tested pigtail macaque (Mn)iPSCs, as a scalable, clinically relevant nonhuman primate model. MniPSCs genetically modified to express P140K had stable transgene expression with 30-fold higher MGMT expression levels compared to untransduced MniPSCs and 15-fold higher expression compared to levels observed in human peripheral white blood cells. P140K-MniPSCs gave rise to chemoresistant $CD34^+$ hematopoietic progenitors that maintained hematopoietic colony forming potential despite treatment with O6BG and bis-chloroethylnitrosourea (BCNU). P140K- and GFP-MniPSCs differentiated into hematopoietic progenitors (50% $CD34^+$) with a predominant long-term (LT)-HSC-like phenotype ($CD34^+CD38^-Thy1^+CD45RA^-CD49f^+$). $CD34^+$ cells expanded on $E4^+$ECs in the presence of StemRegenin 1 (SR1) maintained hematopoietic colony forming potential relative to unexpanded controls, in contrast to cells expanded +/- SR1 or $E4^+$ECs alone. These conditions supported a 22-fold increase in $CD34^+$ cell content and 10-fold increase in MniPSC-hematopoietic progenitors displaying an LT-HSC phenotype. P140K- and GFP-MniPSC-$CD34^+$ cells expanded in this co-culture system exhibited stable engraftment in NSG mice, at levels comparable to Mn bone marrow derived $CD34^+$ cell engrafted mice (up to 10% nonhuman primate $CD45^+$

cells in blood, with myeloid and lymphoid subsets represented). Treatment with O6BG and BCNU increased engraftment up to 6-fold in mice transplanted with P140K-MniPSC CD34$^+$ cells expanded on E4$^+$ECs, but did not increase engraftment in mice that received P140K-MniPSC CD34$^+$ cells expanded without E4$^+$ECs. Mild myelosuppression was observed after O6BG/BCNU treatment. Untreated control mice transplanted with nonhuman primate bone marrow derived CD34$^+$ cells or GFP-MniPSC CD34$^+$ cells alone maintained or lost engraftment over time. Together, these data show that P140K expression in combination with O6BG/BCNU chemotherapy support in vivo selection of MniPSC-CD34$^+$ cells expanded on E4$^+$ECs. Studies are currently underway to determine how P140K and the developmental cues provided by E4$^+$ECs converge to promote engraftment of PSC-derived cells in vivo. These studies highlight a novel and significant advance toward clinical translation of PSC-based blood therapeutics and the development of a nonhuman primate preclinical model.

F-1172

PROGRESS TOWARDS IN VITRO RED BLOOD CELL PRODUCTION

Joanne C. Mountford[1], Emmanuel N. Olivier[1], Angela McCahill[1], Alison Condie[1], Lamin Marenah[2], Aidan Courtney[3], Paul de Sousa[4], Lesley M. Forrester[5], David J. Anstee[6], William G. Murphy[7], Marc L. Turner[8]
[1]ICAMS, University of Glasgow, Glasgow, United Kingdom, [2]Scottish National Blood Transfusion Service, University of Glasgow, United Kingdom, [3]Roslin Cell Ltd, Edinburgh, United Kingdom, [4]Roslin Cells Ltd, Edinburgh, United Kingdom, [5]MRC Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, United Kingdom, [6]Bristol Institute for Transfusion Sciences, NHS Blood & Transplant, Bristol, United Kingdom, [7]National Blood Transfusion Programme, Health Service Executive, Dublin, Ireland, [8]Scottish National Blood Transfusion Service, Edinburgh, United Kingdom

.
Blood transfusion is a wide spread and important clinical intervention, however problems persist both nationally and internationally in maintaining adequacy of supply, managing the risk of transmission of infectious agents and immune incompatibility between donor and recipient. Human embryonic and induced pluripotent stem cells (hESCs & hiPSC) have unique properties in that they can be maintained indefinitely in culture in an undifferentiated state and yet retain the ability to form all the cells and tissues within the body. They therefore offer a potentially scalable source from which to generate red cells (RBCs) for use in clinical transfusion.
We have evaluated hESC lines derived under Good Manufacturing Practice (GMP) conditions in compliance with UK regulatory requirements for clinical products and are preparing master cell bank stocks of the lead line for clinical use, RC9. We are also able to differentiate these RC9-hESCs to form haematopoietic progenitor cells (HPC) which subsequently result in ≥95% conversion to erythroid cells (GlyA+, CD45-, haemoglobinised) with up to 350,000 fold expansion in cell numbers, in a stroma-free, animal product-free suspension based culture system. The culture process starts with a short embryoid body stage followed by sequential changes in inductive cytokines and growth factors taking up to 30 days. We have also demonstrated that this methodology is similarly effective for hiPSC. The erythroid cells express foetal (alpha/gamma) rather than embryonic (epsilon/zeta) haemoglobin but enucleation rates remain low at <5%.
Many challenges exist in taking this product through to clinical trial including improving the rate of enucleation, scale-up and optimisation of culture process, cGMP-translation and cost-reduction in manufacturing and the quality and regulatory challenges of mounting first in man clinical studies.

F-1174

EXTRACTING MECHANISMS FOR HEMATOPOIETIC STEM CELL HOMING TO THE NICHE - A COMPARISON OF IN VIVO AND IN SILICO DATA

Axel Krinner[1], Mark Scott[2], Cristina Lo Celso[2], Tilo Pompe[3], Nico Scherf[1], Ingo Roeder[1]

[1]Institute for Medical Informatics and Biometry, TU Dresden, Dresden, Germany, [2]Department of Life Sciences, Imperial College London, London, United Kingdom, [3]Institute of Biochemistry, Universität Leipzig, Leipzig, Germany

For decades the bone marrow has been recognized as the home of hematopoietic stem cells. Their regulation has been studied extensively, in particular regarding single molecules and pathways, but a comprehensive and dynamic picture is still elusive. This holds especially true for spatial organization. Recent advances in imaging techniques now allow acquisition of data on position and migration in vivo. However, those techniques are limited by a relatively small observation volume and time span due to experimental limitations.
Here, we complement in vivo imaging by a mathematical model of spatio-temporal HSC dynamics and exploit its potential to identify basic principles of stem cell / niche organization. The computer model allows us to test a number of general mechanisms of HSC / niche interaction independent of molecular details. In the work presented, we restrict ourselves to various modes of cell migration and geometries of niche distribution. Cell migration modes cover random (Brownian) motion as observed in vitro, reduction of motility within the niche, as well as attraction and repulsion mechanisms. The structures of niche distributions vary depending on regularity of niche distribution, niche density, and radius of attraction/repulsion areas.
In vivo experiments in mice have yielded data on the distances of transplanted hematopoietic cells to two key structures of the niche: osteoblasts and bone surface. Transplantation of hematopoietic stem and various types of progenitor cells resulted in different distributions of distances to osteoblast. This in vivo data has been compared to in silico results. Interestingly, simulated distance distributions of various hematpopoietic cell types to osteoblasts turned out to be most similar to experimental data for random motion and not for an explicit niche-mediated attraction. A possible explanation of this result might be that at the time of imaging the homing process has not been completed yet. Considering the early in vivo imaging time (i.e. 0.5-5h after injection) we, therefore, expect the homing process in vivo to continue until converging to a final distribution.
The in silico model predicts the spatial cell distributions to depend on different mechanisms. Increasing attraction towards the niche results in an increasing probability of vanishingly small distances. Explicit attraction and repulsion from occupied niches reduce cells at moderate distances. Finally, a less pronounced reduction of motility within the niche leads to a broader distribution depending on the ratio of attraction and motility. Such a theoretical analysis will help to narrow down the spectrum of molecular mechanisms involved in homing and lead to a better understanding of homing and stem cell maintenance.

F-1175

A STEM CELL INTRINSIC SWITCH FROM CANONICAL TO NON-CANONICAL WNT SIGNALLING REGULATES AGING OF HEMATOPOIETIC STEM CELLS

Maria Carolina Florian[1], Kalpana Nattamai[3], Karin Doerr[1], Gina Marka[1], Hans Kestler[2], Karin Scharffetter-Kochanek[1], Yi Zheng[3], Hartmut Geiger[1]
[1]University of Ulm, Ulm, Germany, [B]Bioinformatics and Systems Biology, [1]University of Ulm, Ulm, Germany, [2]Cincinnati Children's Hospital Medical Center and University of Cincinnati, Cincinnati, OH, United States

Somatic stem cell aging is one underlying cause of age-related tissue attrition and disease. Aging of hematopoietic stem cells (HSCs) is associated with impaired hematopoiesis in the elderly. Despite a large amount of data describing the decline of HSC function upon aging, the molecular mechanisms of this process remain still largely unknown. This precludes rational approaches to attenuate stem cell aging. Aged muscle stem cells, in which canonical Wnt signalling is elevated, can be activated to differentiate and regenerate muscles in aged mice as efficiently as young muscle stem cells either by forced activation of Notch, or by Wnt inhibitor factors in serum from young animals supplied by parabiosis. Whether there is a similar critical role of Wnt signalling in aging of HSCs remains largely unexplored.
We determined expression levels of several Wnt mRNAs in young (from 10-12 week-old mice) and aged (24-26 month-old mice) HSCs by real time RT-PCR. Surprisingly, aged HSCs (LIN-, Sca-1+, c-Kit+, CD34-, flk2-) presented with high levels of expression of Wnt5a and Wnt4, primarily associated with non-canonical Wnt signalling. In con-

trast, the expression of Wnt1, 3a, 5b and 10b, which are associated with canonical Wnt signalling, was not altered upon HSC aging. Axin2, a direct downstream target of canonical Wnt signalling was markedly decreased in aged HSCs alongside with reduced expression and reduced nuclear localization of β-catenin, implying an inhibitory role for elevated non-canonical Wnt signalling on canonical Wnt signalling upon HSC aging.
We recently reported that the majority of young HSCs are polar for a large number of planar cell polarity proteins and tubulin as well as for acetylated Histone 4 at Lysine16 (AcH4K16) within the nucleus, while the majority of aged HSCs are apolar. Implying a causal role for Wnt5a in aging of HSCs, in vitro treatment of young HSCs with Wnt5a induced (1) aging associated stem cell apolarity (loss of polarity measured for AcH4K16, Cdc42, tubulin and NCam2), (2) reduction of regenerative capacity and (3) an aging-like myeloid-lymphoid differentiation skewing via activating the small RhoGTPase Cdc42. Conversely, Wnt5a haploinsufficiency (Wnt5a+/- 24 month-old C57/Bl6 mice; Wnt5a-/- mice die perinatally) attenuated HSC aging (restoration of HSC polarity, increased level of B-cell and reduction of myeloid cells in peripheral blood, increased red blood cell count and haemoglobin levels) while stem cell intrinsic reduction of Wnt5a expression by a knock down approach in already aged HSCs resulted in functionally rejuvenated aged HSCs in transplantation experiments.
In summary, our data demonstrate an unexpected shift from canonical to non-canonical Wnt signalling upon aging of HSCs induced by elevated Wnt5a expression in aged HSCs and that inhibition of Wnt5a reverts aging of HSCs. Mechanisms that result in the induction of Wnt5a expression in aged HSCs possibly involve epigenetic modifications, as it has been described that Wnt5a expression is malleable by epigenetic regulation.

F-1177

BONE CELLS GOVERN T LYMPHOPOIESIS BY REGULATING THYMIC EMIGRANTS FROM BONE MARROW

Vionnie W. C. Yu[1], Sutada Lotinun[2], Borja Saez[1], Colleen Cook[1], Rushdia Z. Yusuf[1], Francesca Ferraro[1], Stefania Lymperi[1], Marc Raaijmakers[3], Joy Wu[4], Henry M. Kronenberg[4], Roland Baron[2], David T. Scadden[1]
[1]Harvard Stem Cell Institute, Harvard Department of Stem Cell & Regenerative Biology, Massachusetts General Hospital, Boston, MA, United States, [2]Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, MA, United States, [3]Department of Hematology and Erasmus Stem Cell Institute, Erasmus University Medical Center, Rotterdam, Netherlands, [4]Endocrine Unit, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States

Mesenchymal cell direction of parenchymal cell activity is hypothesized to be critical for adult tissue function, but remains poorly defined. We examined how specific subsets of osteolineage mesenchymal cells affect parenchymal hematopoietic cells in the bone marrow by a method of selective cell depletion. Unexpectedly, mature bone cells expressing osteocalcin (Ocn+) were found to be critical for T lymphopoiesis through the modulation of cells destined for thymic emigration. Specific depletion of Ocn+ cells reduced the number of adult bone marrow T-lymphoid biased progenitors by reduced endosteal DLL4 production and hematopoietic progenitor Notch activation. Thymic emigrants were compromised in association with reduced CCR7 expression, yet were capable of normal T lineage differentiation upon adoptive transfer to the thymus. B cell, myeloid progenitor cell and hematopoietic stem cell numbers were unperturbed. Therefore, mature osteolineage cells have a highly constrained hematopoietic cell specific effect, altering T cell production by regulation of thymic emigrants and their ability to translocate to the site of maturation. These data suggest a new role for bone in the homeostasis of the immune system.

F-1178

E2F1 IS CRITICAL FOR SURVIVAL OF CHRONIC MYELOID LEUKAEMIA **(**CML) STEM CELLS

Francesca Pellicano[1], Lisa Hopcroft[1], Laura Park[1], Amy Sinclair[1], Gustavo Leone[2], Mark Girolami[3], Anthony Whetton[4], Kamil Kranc[1], Tessa Holyoake[1]

[1]Paul O'Gorman Leukaemia Research Centre, University of Glasgow, Glasgow, United Kingdom, [2]The Ohaio State University, Columbus, OH 43210, OH, United States, [3]Department of Statistical Science, UCL, London, United Kingdom, [4]University of Manchester, Manchester, United Kingdom

CML is a myeloid malignancy arising at the haemopoietic stem cell (HSC) level induced by the oncogenic *BCR-ABL* tyrosine kinase. CML LSC are not BCR-ABL kinase dependent and may rely on a number of alternative survival pathways. We carried out global gene expression profiling between quiescent (G0) normal HSC and CML LSC and observed that CML LSC are transcriptionally more prone to proliferation compared to their normal counterparts, through an unknown mechanism. A key finding was that G0 LSC, but not normal HSC, showed up-regulation of several members of the cell cycle machinery, including the transcription factor E2F1. Increased activity of E2F1 in primitive $CD34^{+}38^{-}$ CML stem cells was suggested by up-regulation of its target genes, including CDC20, Cyclin B2, CDK1, CHEK1, CDC25C, CDC6 and CDC27. E2F1 was knocked-down in primary CML and normal $CD34^{+}38^{-}$ cells. After knock-down, $CD34^{+}38^{-}$ cells showed a 50% decrease in E2F1 mRNA and 45% decrease in E2F1 protein. We measured proliferation in stem/progenitor cells by tracking individual cell divisions. Decreased levels of E2F1 caused a block in proliferation in CML but not in normal stem/progenitor cells. We observed that knock-down of E2F1 impaired CML LSC function, leading to a decrease in CFC numbers and an increase in cell death by apoptosis. We then carried out genome-wide miRNA expression profiling and OncoMir MicroRNA Q-PCR using CML and normal $CD34^{+}38^{-}$ cells. Data analysis revealed several potential candidates that are known to be deregulated in cancer, including the polycistronic cluster for hsa-mir183/182/96. Hsa-mir183 was up-regulated 38-fold inCML versus normal $CD34^{+}38^{-}$ cells and computational target prediction showed that early growth response 1 (EGR1) was a high scoring putative target for hsa-mir183. Interestingly EGR1 is a key regulator of E2F1 and Q-PCR analysis showed that EGR1 was down-regulated in CML versus normal $CD34^{+}38^{-}$ cells. Following knock-down of hsa-mir183 in CML $CD34^{+}38^{-}$ cells by a miRZip-shRNA lentiviral-based system the level of transcription of EGR1 was increased. Knock-down of hsa-mir183 also caused a decrease in CML $CD34^{+}38^{-}$ cell proliferation and CFC analysis showed a decrease in myeloid colony forming ability. The investigation of the role of E2F1 in normal HSC and CML LSC functions *in vivo* is currently on-going. We characterised normal haemopoiesis in E2F1 knock-out (KO) mice. Using immunophenotypic analysis we observed that the total numbers of long-term (LT)-HSC were decreased in E2F1-KO mice. In addition, the ability of E2F1-KO stem/progenitor cells to form multilineage colonies in CFC assays was decreased. We are now in the process of determining the impact of E2F1 deletion on the development and maintenance of CML. In conclusion, we have observed that CML LSC are transcriptionally more prone to proliferation compared with their normal counterparts. This process is likely to be caused by increased E2F1 transcription and activity mediated by hsa-mir183 and its target genes. E2F1, by deregulating specific LSC-intrinsic mechanisms, appears to be required for CML stem cell survival *in vitro* and its knock-down or deletionare anticipated to both block CML development and lead to LSC death.

F-1181

AN INDUCED PLURIPOTENT STEM CELL-BASED PLATFORM FOR IN VITRO STUDY OF CHRONIC MYELOID LEUKEMIA STEM CELL DEVELOPMENT AND DRUG RESISTANCE

Kran Suknuntha[1], Yuki Ishii[2], Kejin Hu[3], Ron Stewart[4], Jean Wang[2], James Thomson[4], Igor Slukvin[1]
[1]Pathology and Laboratory Medicine, UW-Madison, Madison, WI, United States, [2]University of California, San Diego, CA, United States, [3]Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL, United States, [4]Morgridge Institute for Research, Madison, WI, United States

Chronic myeloid leukemia (CML), a myeloproliferative disorder characterized by unregulated growth of predominantly myeloid cells and their accumulation in the bone marrow and peripheral blood, originates in hematopoietic stem cells (HSCs) with t(9;22)(q34;q11.2) translocation which produces Bcr-Abl fusion transcript. An Abl tyrosine kinase inhibitor (TKI), imatinib, employed as a first line of treatment for CML is able to induce complete cytogenetic

response in most of the patients. However, it does not cure CML due to persisting leukemic stem cells (LSCs). Therefore, eradication of CML requires understanding the mechanisms of imatinib resistance and developing drugs targeting alternative cell survival pathways in CML LSCs. Due to a very low number of BCR-ABL+ cells within the most primitive hematopoietic cell compartments compared to dominance of leukemic cells in more mature and terminally differentiating compartments, study in LSCs is limited. Here we used induced pluripotent stem cells (iPSCs) to generate lin-CD34+CD45+ in vitro and determine whether these iPSCs-derived cells can be used to screen for new drug targets to eliminate CML LSCs. We found that lin-CD34+CD45+ primitive hematopoietic cells generated from CML iPSCs display LTC-IC potential, high ALDH activity, and efflux rhodamine. These cells express functional Bcr-Abl with kinase activity (pCRKL) compared to normal iPSCs. Besides hematopoietic stem cell properties, the CML iPSCs-derived cells display distinctive LSC features, including adhesion defect, imatinib resistance, and rapid limited cytokine independent proliferation. Following maturation expansion with cytokines, lin-CD34+CD45+ cells lost CD34 expression and regained sensitivity to imatinib. All together, these data suggest that lin-CD34+CD45+ cells obtained from CML-iPSCs represent functional equivalent of LSCs. Comparative analysis of gene expression in CML-iPSC lin-CD34+CD45+ cells treated and not treated with imatinib identified olfactomedin 4 (OLFM4) as the top-ranking gene among 34 genes that were induced by imatinib. OLFM4 knockdown using siRNA increased apoptosis in vitro of lin-CD34+CD45+ cells from CML iPSCs. The effect was enhanced when cells were treated with imatinib, indicating that OLFM4 may represent a potential novel drug target for somatic CML-LSCs. To confirm our hypothesis, we performed OLFM4 knockdown in lin-CD34+ from a parental CML sample. We found that similar to iPSC-derived LSCs, OLMF4 knockdown in somatic LSCs significantly increased apoptosis and reduced colony-forming activity. Thus, identification of primitive hematopoietic cells with LSC properties in iPSC cultures provides an iPSC-based platform to study leukemia stem cell development and mechanisms of drug resistance in a patient-specific manner.

F-1182

GENERATION OF PLATELETS IN A SERUM AND FEEDER-FREE ENVIRONMENT.

Marjorie Pick[1], Ihab Abd-Elrahman[1], Lisa Azzola[2], Riki Perlman[1], Andrew Elefanty[3], Dina Ben Yehuda[1]
[1]Department of Hematology, Sharett Institute, Jerusalem, Israel, [2]Monash Immunology and Stem Cell Laboratories, Victoria, Australia, [3]Murdoch Childrens Research Institute, Victoria, Australia

Background: The production of human platelets *in vitro* in a defined culture system is a prerequisite for the generation of platelets for therapeutic use. As an important step towards this goal, we report the differentiation of human stem cells (hSCs) towards polyploid megakaryocytes (Mk) and robust platelet production by supplementing a novel pro-apoptotic protein into a serum- and feeder-free differentiation medium. Endoreplication is the process that allows Mks to duplicate their DNA to generate platelets. The endomitotic cell cycle consists of an S phase interrupted by a gap, during which the cells enter mitosis but skip anaphase B and cytokinesis and thus do not divide into two cells but multiply their DNA. It is believed that Mks undergo a specialized form of apoptosis to shed platelets after endomitosis is complete. However, to do this they do not activate the intrinsic apoptotic pathway to generate platelets; rather, they restrain it to survive and progress safely through proplatelet formation and platelet shedding. We have found a novel function of an apoptotic protein that once cleaved has pro-apoptotic activity that enables Mks to generate robust levels of platelets *in vitro*.
Results: Immunophenotypic analyses of differentiating human embryonic stem cells identified a subpopulation of cells expressing high levels of CD41a that expressed other markers associated with the Mk lineage, including CD110, CD42b and CD61. $CD41^{+/dim}$ cells contained most of the myeloid progenitors that generated mixed, erythroid and megakaryocytic colonies at both day 13 and day 20 of differentiation. Furthermore, $CD41a^{+}$ cells had ability to endoreplicate DNA and showed more than 2 copies of each chromosome. These studies were complemented by real time PCR analyses showing that subsets of cells enriched for $CD41a^{+}$ Mk precursors expressed significantly higher levels of Mk associated genes such as *PF4* and *MPL*. Differentiated Mk cells produced platelet-like particles that expressed CD42b and were activated by ADP, similar to platelets from cord blood. We further studied the ability of primary Mks generated from $CD34^{+}$ cord blood cells over-expressing a protein that regulates apoptosis to produce platelets. After 14 days in culture containing thrombopoietin and stem cells factor, the Mks generated

had the capacity to endoreplicate to a higher ploidy and produced 7 times more CD41a$^{=}$ functional platelets then CD34^{+} cells containing an empty vector control.
Conclusions: Here we have described a serum- and feeder-free culture system that enabled the generation of functional platelets and Mk progenitors from human stem cells. The differentiated Mks fragmented to form platelet-like particles. This protocol represents an important step towards the generation of human platelets for therapeutic use. Moreover, over-expression of a regulator of apoptosis in CD34^{+} cells induced differentiation of these cells into Mks and increased their ability to produce platelets.

F-1183

LOSS OF ARYL HYDROCARBON RECEPTOR SIGNALING IN HEMATOPOIETIC CELLS PROMOTES CHANGES IN GENE EXPRESSION ASSOCIATED WITH HEMATOPOIETIC STEM CELL FUNCTION

John A. Bennett, Kameshwar P. Singh, Thomas A. Gasiewicz
University of Rochester, Rochester, NY, United States

Loss of Aryl Hydrocarbon Receptor Signaling in Hematopoietic Cells Promotes Changes in Gene Expression Associated with Hematopoietic Stem Cell Function
JA Bennett, KP Singh and TA Gasiewicz
The Aryl hydrocarbon receptor (AhR) is a ligand activated bHLH transcription factor belonging to the Per-Arnt-Sim (PAS) superfamily, a family of proteins involved in mediating the response to cellular environment such as changes in oxygen availability as well as regulation of circadian rhythms. AhR plays a major role in mediating the toxic and carcinogenic effects of many environmental pollutants. Though our understanding of the physiological roles of the AhR in the immune system is evolving, there is little known about its role in hematopoiesis and hematopoietic diseases. Epidemiological studies have linked exposure to xenobiotic AhR ligands such as polychlorinated biphenyls and polychlorinated dibenzodioxins/furans with increased incidence of non-Hodgkins lymphoma and myeloid leukemia. We hypothesized that lifetime exposure to environmental pollutants that accumulate with age and affect AhR expression/activity may contribute to abnormal hematopoiesis and hematopoietic disease. Studies in our lab have indicated that AhR null-allele (AhR-KO) animals are impaired in hematopoietic stem cell function and develop myeloproliferative changes in peripheral blood, as they age. Young AhR-KO mice have alterations in hematopoietic stem and progenitor cell populations in the bone marrow. We hypothesized that a mouse strain (cKO) lacking AhR expression specifically within hematopoietic cells would develop similar changes, as they age. However, we have not observed a complete phenocopy of the AhR null–allele animals at early ages. In order to illuminate the signaling mechanisms underlying the alterations in hematopoiesis observed in the AhR-KO mice, we sorted a population of cells highly enriched for HSC function (LSK CD34- CD48- CD150+) and performed microarray analyses. In order to determine what the changes in gene expression may underlie the development of the phenotype observed in aging animals, we chose to analyze sorted HSCs from young/old AhR-null allele and cKO mice. Ingenuity Pathway Analysis / Gene Set Enrichment Analysis was performed on the microarray data, revealing that there are alterations in networks of genes important for hematopoietic stem cell function, engraftment and development of leukemia or other hematological diseases in both knockout strains. Interestingly, many genes were only altered in one strain. Only a small subset of affected genes was shown to overlap between the two strains, and even fewer of these genes (such as Stra13, Fam164c and Vmn1r218) were simultaneously up/downregulated in both strains. This data suggests that loss of AhR alters signaling networks in hematopoietic cells in a cell-intrinsic fashion, but also suggests a role for AhR-mediated stromal cell signaling having an effect on the signaling pathways activated in the HSC population in bone marrow. This may occur through alterations in contact–mediated signaling or soluble paracrine/endocrine factors. More work is needed to define what these signals may be, and how they act upon hematopoietic stem cells to produce altered function and phenotype. Supported by NIH Grants P30 ES01247, T32 ES07026, and ES04862.

F-1184

METABOLIC REGULATION OF HEMATOPOIETIC STEM CELL AND HEMATOLOGICAL MALIGNANCY

Ying-Hua Wang[1], William Israelsen[2], Dong-Jun Lee[1], Clary Clish[3], Matthew Vander Heiden[2], David Scadden[1]
[1]Stem Cell and Regenerative Biology, Harvard University & Massachusetts General Hosp, Boston, MA, United States, [2]David Koch Institute for Integrative Cancer Research, MIT, Cambridge, MA, United States, [3]Broad Institute of MIT and Harvard, Cambridge, MA, United States

It has been increasingly realized that different cell states employ different metabolic modes. Whether the metabolic mode is a determinant of cell state is unclear, but studies in cancer cells suggest that metabolic shifting from glycolysis toward oxidative phosphorylation can modify in vitro and in vivo function of tumorogenic cells. Cancer cells metabolize the majority of glucose into lactate even in the presence of oxygen, a phenomenon known as aerobic glycolysis or the Warburg effect. Normal somatic cells thought to also preferentially use anaerobic metabolism are tissue stem cells, particularly the self-renewing hematopoietic stem cells (HSC) resident in the hypoxic microenvironment of the bone marrow. Recently the molecular basis for the Warburg effect in cancer cells has been identified as due to a specific isoform of pyruvate kinase M2 (PKM2). In contrast, normal tissues that rely on oxidative phosphoryation express the alternatively spliced isoform PKM1. Interestingly, we observed that cells in the hematopoietic lineage, including HSC, predominantly express PKM2. To understand the role of glycolytic metabolism in HSC as well as in hematological malignancy, we have developed a genetically modified mouse strain that allows conditional deletion of PKM2. Our data show deletion of PKM2 leads to upregulation of PKM1, accompanied with decreased glycolysis and increased oxidative phosphorylation specifically in the hematopoietic stem/progenitor population. Under homeostatic conditions, no apparent changes in normal hematopoiesis were observed in $PKM2^{-/-}$ mice. Loss of PKM2, however, appears to compromise the long-term repopulation capacity of HSC as revealed by serial transplantation assay. Moreover, we also observed that deletion of PKM2 markedly disadvantaged the establishment of leukemia in mice when hematopoietic cells express human leukemogenic alleles associated with either acute or chronic myeloid leukemia. The impact of PKM2 depletion on hematopoiesis and leukemia development is associated with defects in biomass synthesis, which is essential for cell to proliferate. Interestingly, we observed very similar phenotypes in mice with deletion of lactate dehydrogenase A (LDAa) in hematopoietic cells. Collectively, these data suggest important roles of glycolytic metabolism in both normal hematopoiesis and leukemogenesis.

F-1185

IDENTIFICATION OF CRITICAL FACTORS AFFECTING MICROGLIA RECONSTITUTION FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR DESIGNING THERAPEUTIC APPROACHES

Alessia Capotondo[1], Rita Milazzo[3], Francesca Cecere[1], Letterio Salvatore Politi[4], Angelo Quattrini[5], Alessia Palini[2], Fabrizio Benedicenti[1], Elia Stupka[6], Eugenio Montini[1], Luigi Naldini[1], Alessandra Biffi[1]
[A]Division of Regenerative Medicine, Stem Cells and Gene Therapy, [B]Flow cytometry Resource Analytical Cytology Technical Applications Laboratory, [1]San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Milano, Italy, [2]Division of Regenerative Medicine, Stem Cells and Gene Therapy, San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Vita-Salute University, Milano, Italy, [A]Imaging Core, Neuroradiology Unit, Head and Neck Department, [B]Division of Neuroscience, [C]Center for Translational Genomics and Bioinformatics, [3]San Raffaele Scientific Institute, Milano, Italy

A critical need exists to enhance and fasten microglia turnover with donor cells following Hematopoietic Cell Transplantation (HCT) in order to anticipate the time of clinical benefit and improve the efficacy of the transplant in severe diseases like Lysosomal Storage Disorders (LSDs). For these disorders reconstitution of microglia with donor-derived elements is of particular value being these cells the most abundant among the myeloid brain populations, thus the most valuable source for metabolic correction of the surrounding tissue. However, obtaining microglia reconstitution by the donor is a challenging goal, particularly when clinically predictive settings are employed. Moreover, very little is known on the modalities of microglia turnover in physiological and pathological conditions, which may help in adequately designing therapeutic approaches. We investigated the modalities of microglia reconstitution after hematopoietic stem and progenitor cell (HSPC) transplantation, tracking a kinetic of the myel-

oid infiltration into the brain, in basal conditions or after the administration of different preparative regimens. By studying wild type and LSD mice at diverse time-points following HCT we showed the occurrence of a short-term wave of brain infiltration by a fraction of the transplanted hematopoietic progenitors, independently from the administration of a preparatory regimen and from the presence of a disease state in the brain. However, only the use of a conditioning regimen capable of ablating functionally-defined brain-resident myeloid precursors allowed turnover of microglia with the donor, mediated by local proliferation of early immigrants rather than entrance of mature cells from the circulation. This model has raised relevant issues that we are addressing. As first, a detailed characterization of bona fide microglia progenitors selectively ablated by the conditioning regimen applied is of great biological relevance. To this goal, we are using the Cd11b-TK mouse model, in which selective conditional ablation of proliferating myeloid cells and microglia could be achieved upon Gancyclovir (GCV) administration. By intracerebroventricular delivery of GCV, preliminary data showed the typical change within the myeloid brain compartment composition observed after the administration of BU, suggesting that possibly GCV was capable to act on the same pool of cells that we could ablate with BU. Secondly, the identification of the fraction within HSPC capable of short-term brain homing and local proliferation would be important for optimizing transplant approaches for LSDs with neurological involvement. For this purpose, we are transplanting different sub-populations purified from the bone marrow HSPC pool, starting from KSL cells, highly enriched in stem cell activity, and their counterpart. We showed that at 6 months post-transplant only KSL cells retain the capability of long-term microglia reconstitution. We are now investigating the capability of microglia reconstitution by KSL sub-populations that differ for stemness and commitment, identified according to the expression of CD150 and CD 48 markers. Preliminary data suggest a different potential of brain microglia reconstitution by the different sub-populations.

F-1186

MIR-126 CONTROLS HEMATOPOIETIC STEM CELL FUNCTION

Francesco E. Boccalatte[1], Bernhard Gentner[1], Alice Giustacchini[1], Massimo Saini[1], Umberto Restuccia[2], Jose M. Garcia Manteiga[3], Eric R. Lechman[4], Peter Van Galen[4], Elia Stupka[3], Angela Bachi[2], John E. Dick[4], Luigi Naldini[1]
[1]Telethon Institute for Gene Therapy, Milan, Italy, [A]Biomolecular Mass Spectrometry unit, [B]Center for Translational Genomics and Bioinformatics, [2]San Raffaele Scientific Institute, Milan, Italy, [3]Ontario Cancer Institute, University Health Network, Toronto, ON, Canada

MicroRNAs (miRNAs) are known to have a regulatory role in hematopoietic stem cell (HSC) lineage commitment and differentiation. We recently demonstrated that miR-126 has high biological activity in hematopoietic stem cells (HSC), which is progressively lost during early stages of differentiation. In order to investigate the function of miR-126 in hematopoietic stem and progenitor cells (HSPCs), we stably transduced human cord blood (huCB)-derived CD34+ cells with miR-126 knockdown- (KD) or overexpressing (OE) lentiviral vectors (LV). In vitro culture in serum-free HSPC maintenance conditions revealed a marked increase in total cell output upon miR-126 KD. This effect was mediated by a specific promotion of the G2/M cell cycle phase, particularly prominent in the more primitive CD34+133+38- subpopulation, and paralleled by an increased content of colony forming cells as assayed in methylcellulose. Most importantly, we demonstrate by competitive transplantation studies that miR-126 KD expands the in vivo pool size of mouse and human HSC without exhausting them, whereas HSC overexpressing miR-126 were progressively lost from the BM due to an impairment in cell cycle entry. Taken together, these data suggest that HSC pool size is influenced by endogenous levels of miR-126 through modulation of the HSC quiescence/proliferation equilibrium, and we are currently exploring the potential of miR-126 antagomirs to expand HSC numbers for cell and gene therapy applications.
To dissect the molecular pathways underlying these biological effects, we performed gene expression profiling on sorted CD34+38- huCB HSPC after transduction with miR-126 KD-, miR-126 OE- or control LVs. Bioinformatic analysis of differentially expressed transcripts pointed to the PI3K/AKT axis as one of the major signal transduction pathways controlled by miR-126 in HSPC. We identified p85B and CRKII as direct miR-126 targets in CB CD34+38- cells, and show enhanced PI3K pathway activation as reflected by increased AKT and GSK3β phosphorylation upon 126 KD, while 126 OE caused opposite effects. Pharmacologic inhibition of p85B reverted the proliferative advant-

age conferred by miR-126 KD, thus confirming the pivotal role of the PI3K/AKT pathway in promoting miR-126 KD driven HSPC expansion.
To identify additional miR-126 targets in primary HSPC, we performed (1) whole RNA sequencing (RNAseq) on huCB CD34+133+38- cells and (2) quantitative proteomics using dimethyl labeling of protein lysates after miR-126 gain- and loss of function. RNAseq identified 153 differentially expressed genes which showed a highly significant enrichment in the GO categories of cell metabolism, cytoskeleton rearrangement and motility (all upregulated upon miR-126 KD), while proteomics revealed changes in proteasome and specific ribosomal protein subunits, in particular those involved in erythroid differentiation (RPS19 and RPL11), thus confirming an impact of miR-126 on HSC fate, RNA processing and cell metabolism. Studies addressing the role of miR-126 in HSC motility, homing and mobilization are underway and will be presented at the meeting. In summary, our data suggest a central role for miR-126 in regulating multiple aspects of HSC biology.

Endothelial Cells/Hemangioblasts

F-1191

IMPROVING THE EXPANSION AND DIFFERENTIATION OUTCOME OF HUMAN UMBILICAL CORD BLOOD DERIVED ENDOTHELIAL PROGENITOR CELLS AND FETAL MESENCHYMAL STEM CELLS THROUGH EX VIVO CULTURE UNDER CONTINUOUS HYPOXIA

Yuchun Liu[1], Swee-Hin Teoh[1], Nuryanti B. Johana[2], Mahesh Choolani[2], Jerry KY Chan[2]
[1]School of Chemical and Biomedical Engineering , Division of Bioengineering, Nanyang Technological University, Singapore, Singapore, [2]Obstetrics and Gynaecology, National University of Singapore, Singapore, Singapore

Our earlier coculture studies combining human umbilical cord blood derived endothelial progenitor cells (UCB-EPC) with human fetal mesenchymal stem cells (hfMSC) for bone tissue engineering applications have highlighted the potency of EPC as a potential vasculogenic and osteogenic-enhancing cell source for stem cell therapy. To further enhance their therapeutic potential, low oxygen tensions of 2% were introduced during ex vivo culture and maintained throughout the entire culture duration. By mimicking the low oxygen tensions of these progenitors in their natural physiological bone marrow niche, we hypothesised it is a critical parameter for maintaining their stem cell phenotype, thereby facilitating the therapeutic vasculogenic and fracture repair response in vivo. Here, our hypoxia investigations were confined to evaluating the growth and differentiation capabilities of individual monocultures due to the complexity of coculture models.
Mononuclear cells harvested from UCB showed early emergence of EPC colonies by Day 10 under 2% hypoxia, forming a large colony of early EPC as compared to none under 21% oxygen tensions after three weeks. Exposure of EPC to continuous hypoxia also led to enhanced growth kinetics, with 1.3-1.8-fold higher proliferative capacity in the first 7 days of culture ($p>0.05$). In addition, hypoxia conditioning of EPC showed a slight trend towards improved vasculogenic potential, with 1.2-fold higher number of branch points formed overnight after seeding onto Matrigel in vitro ($p>0.05$). This was supported by slight upregulation of VEGF-A and VEGF-C by 1.21 and 2.4-fold respectively ($p>0.05$), whereas other angiogenic genes such as CXCL1, CXCL3, MMP9, TIMP2, TIMP3 was significantly downregulated by 3.2 - 44.0 fold ($p<0.05$) under hypoxia. In a similar approach, bone marrow aspirates were harvested and hfMSC demonstrated more robust osteogenic capacity, with 2.9 fold higher calcium content on Day 14 ($p<0.001$), and increased CFU-F capacity (6.5 fold; $p<0.05$) under hypoxia. Investigations of the vasculogenic and osteogenic potential of EPC and hfMSC under hypoxic priming warrants confirmation in an in vivo paradigm.
This study provides new insight to the importance of maintaining a continuous hypoxic environment upon ex vivo harvest, for the purpose of improving the expansion and differentiation capacity of stem cells. Results have demonstrated potential benefits of the utility of continuous hypoxia upon ex vivo culture for obtaining higher cellular yield for therapeutic use. This is particular so for EPC which have a slow proliferation capacity and low yield using current non-optimal isolation techniques. Future studies of EPC in coculture with hfMSC will be investigated under a hypoxia environment.

F-1192

HUMAN INDUCED PLURIPOTENT STEM CELLS DIFFERENTIATE INTO PURE POPULATIONS OF ENDOTHELIAL CELLS MEDIATED BY SET SIMILAR PROTEIN THROUGH VE-CADHERIN TRANSCRIPTIONAL ACTIVATION

Andriana Margariti[1], Yanhua Hu[2], Alan W Stitt[1], Qingbo Xu[3]

[1]Queen's University Belfast, Belfast, United Kingdom, [2]King's College, London, United Kingdom, [3]King's College London, London, United Kingdom

The generation of induced pluripotent stem cells (iPSCs) have great potential for regenerative medicine as they can differentiate into specific cell lineages and/or be utilised for drug screening and disease modelling. Vascular diseases are a leading cause of mortality in the Western world with initiation and progression of pathology being closely linked to damaged endothelial cells (ECs) on the vessel wall. iPSCs are an attractive option to derive new, fully functional ECs to regenerate damaged vasculature. In this study we have generated iPSCs from human fibroblasts using viral induction of the four reprogramming transcription factors (OCT4, SOX2, KLF4, and c-MYC) or using a DNA-free integration method based on a single plasmid transfection and neomycin selection. iPSCs generated by plasmid transduction and selection was faster and provided a higher efficiency of colony formation. Colonies obtained using both methods were isolated, expanded and fully evaluated according to the standard criteria of iPSCs characterisation, including morphology, pluripotent marker expression, alkaline phosphatase staining, and teratoma formation potential. Human iPSCs were differentiated towards vascular cell lineages and specific to ECs. To this end, human iPSCs were seeded on collagen IV and cultured with differentiation media containing a cocktail of growth factors such as Vascular Endothelial Growth Factor (VEGF). Time course experiments revealed the ability of iPSCs to differentiate into ECs and also revealed a greater potential to derive ECs from pluripotent cells generated by transient transfection. In an attempt to obtain a pure population of differentiated ECs, iPSCs cells were pre-differentiated into an early progenitor stage and CD34 selection was performed. The $CD34^+$ cells were then induced to differentiate into ECs and it was demonstrated that these selected cells provided greater efficiency and led to greater homogeneity in derived ECs, expressing in high levels endothelial markers such as VE-Cadherin, PECAM1, KDR, eNOS and vWF. During iPSCs differentiation into ECs, the gene SETSIP (similar to SET protein) was found to be up-regulated. In order to define the mechanistic role of SETSIP, the protein was over-expressed and this markedly enhanced EC differentiation. Conversely, when SETSIP was knockdown by shRNA, EC differentiation was significantly suppressed. Importantly, immunofluorescence staining showed a nuclear translocation for SETSIP during EC differentiation and subsequent luciferase assays indicated that SETSIP and not SET protein induced VE-Cadherin transcriptional activation in the ECs. In this study we have shown that CD34 positive cells derived from pre-differentiated human iPSCs have the potential to differentiate into ECs. SETSIP appears to have a critical role in this process though control of VE-Cadherin transcriptional activation. Ongoing studies that identify the key mechanisms controlling iPSC-derived EC-differentiation have considerable relevance for regeneration of damaged vasculature in a range of diseases.

F-1193

IPSC-BASED MODELING OF HUMAN NOTCH1 MUTATIONS REVEALS NOVEL PATHWAYS REGULATING CALCIFIC AORTIC VALVE DISEASE

Christina Theodoris, Mark White, Lei Liu, Daniel He, Benoit Bruneau, Deepak Srivastava
Gladstone Institute of Cardiovascular Disease, University of California, San Francisco, San Francisco, CA, United States

In humans, *NOTCH1* mutations result in congenital heart defects including valve malformations and severe valve calcification in adults. To understand the mechanisms by which NOTCH1 mutations in endothelial cells (ECs) cause disease, we generated episomally induced pluripotent stem cell (iPSC) lines from fibroblasts of four individuals from two families affected with aortic valve disease due to heterozygous mutations in *NOTCH1*. As a control, we generated an iPSC line for an unaffected wild-type patient from the same family as two of the affected patients. We differentiated control and mutant iPSC lines into ECs using a differentiation protocol developed in our lab. We exposed the ECs to either static or fluid shear stress conditions to model the aortic or ventricular side of the valve, respectively. NOTCH1 mRNA levels were significantly decreased in the NOTCH1+/- ECs in both static and shear stress conditions. Of the NOTCH1 transcripts sequenced in heterozygote ECs, 75-90% were transcribed from the wild-type copy of NOTCH1, suggesting that the mutant mRNA was likely degraded by nonsense-mediated decay. Initial RNA-seq results indicated that 1118 genes were differentially expressed in static conditions and 1052 genes responded abnormally to shear stress in NOTCH1+/- ECs compared to NOTCH1+/+ ECs. Differentially expressed genes included canonical NOTCH1 targets *HRT2* and *EFNB2* as well as novel targets involved in vascular development, inflammation, and endochondral ossification. Overall, gene ontology terms significantly overrepresented in differentially expressed genes included cardiovascular development and response to wounding, suggesting that the NOTCH1+/- ECs were unable to mount the normal developmental and protective response to shear stress in the valve. The gene networks dysregulated in NOTCH1+/- ECs as determined by NOTCH1 ChIP-seq, differentially methylated regions of DNA, and differences in the progression of chromatin states during EC differentiation will be presented. Determining the consequence of *NOTCH1* heterozygous mutations in human patient-specific ECs will greatly increase our understanding of the role NOTCH1 plays in aortic valve calcification and may reveal novel targets for intervention.

F-1194

HEART FIELD ORIGIN OF GREAT VESSEL PRECURSORS RELIES ON NKX2.5-MEDIATED VASCULOGENESIS

Noelle Paffett-Lugassy[1], Reena Singh[2], Burcu Guner-Ataman[1], Richard P. Harvey[2], C. Geoffrey Burns[1], Caroline E. Burns[1]
[1]*CVRC, Massachusetts General Hospital, Charlestown, MA, United States,* [2]*Victor Chang Research Institute, Darlinghurst, Australia*

The pharyngeal arch arteries (PAAs) are transient embryonic blood vessels that make indispensable contributions to the carotid arteries and great vessels of the heart, including the aorta and pulmonary artery. During embryogenesis, the PAAs appear in a craniocaudal sequence to connect pre-existing segments of the primitive circulation after de novo vasculogenic assembly from angioblast precursors. Despite the unique spatiotemporal characteristics of PAA development, the embryonic origins of PAA angioblasts and the genetic factors regulating their emergence remain unknown. Here, we identify the embryonic source of PAA endothelium as nkx2.5+ progenitors in lateral plate mesoderm long considered to adopt cell fates within the heart exclusively. Further, we report that PAA endothelial differentiation relies on Nkx2.5, a canonical cardiac transcription factor not previously implicated in blood vessel formation. Specifically, we tracked the migratory behavior of nkx2.5+ cells in the bilateral heart field of zebrafish embryos. Surprisingly, we identified a vasculogenic population that condensed into pharyngeal clusters, initiated expression of the angioblast marker tie1, and concomitantly downregulated nkx2.5. Using complementary lineage tracing strategies in zebrafish, we learned that each nkx2.5+ cluster derives a single PAA with a large majority of PAA progenitors stemming from the nkx2.5+ embryonic heart field. Similarly, Cre/loxP lineage tracing in mouse confirmed the evolutionary conservation of PAA endothelial cell derivation from nkx2.5+ progenitors. Loss of function studies in both zebrafish and mice demonstrated that Nkx2.5 is essential for PAA establishment. Further, our

analyses in zebrafish revealed that nkx2.5 is required cell autonomously for PAA endothelial cell differentiation. Together, these studies reveal the heart field origin of PAA endothelium and attribute a novel vasculogenic function to the cardiac transcription factor nkx2.5 during great vessel precursor development.

F-1195

ENHANCEMENT OF AUTOPHAGY WITH HYPOXIC PRECONDITIONING IMPROVES SURVIVAL OF TRANSPLANTAED PROGENITOR ENDOTHELIAL CELLS

Hai-Jie Wang, Yu-Zhen Tan, Pei Zhou
Shanghai Medical School of Fudan University, Shanghai, China

Endothelial progenitor cells (EPCs) have a potential to differentiate toward endothelial cells and participate in angiogenesis of the ischemic tissues. Recently, clinical studies show that transplantation of EPCs is effective for treating myocardial infarction and limb ischemia. However, survival of the transplanted EPCs in ischemic microenvironment is poor. This investigation was designed to examine autophagic effect of hypoxia pretreatment in maintaining survival of the transplanted EPCs. $CD34^{+}VEGFR\text{-}2^{+}$ EPCs isolated from rat marrow were incubated in hypoxic conditions (3% or 1% O_2) for 1, 2, 4 h respectively. The autophagic structures were exemined with MDC staining. Expression of HIF-1 α, beclin-1 and LC3 was evaluated by immunocytochemical staining and western blot analysis. After hypoxic treatment, the autophagic structures in the cells increased, expression of HIF-1 α, beclin-1 and LC3 was enhanced. Lysosomes also increased in hypoxia-exposed cells. Apoptotic cells in hypoxic cells increased when autophagy was inhibited with 3-methyladenine. Moreover, pretreatment with rapamycin improved survival of hypoxia-pretreated cells. The cells pretreated with 3% O_2 for 2 h were selected for transplantation experiment. Rat models of myocardial infarction were established by ligation of the anterior interventricular artery. The EPCs carried with fibrin glue were implanted into the periphery of the infracted region. Distribution of the implanted cells was examined with DiI labeling and Y chromosome fluorescence *in situ* hybridization. After confirming transplanted position on semithin section, the autophagic ultrastructures in transplanted cells were observed with transmission electron microscope. Compatibility of fibrin with EPCs and myocardium was well. Compared with EPCs group, number of the remained cells was greater in EPCs carried with fibrin glue group. After transplantation for 24 h, the survived cells in hypoxia pretreatment group were more than that in control group. At 4 week after transplantation of the hypoxia-pretreated cells, myocardial regeneration and angiogenesis increased and cardiac functions were improved significantly. These results demonstrate that enhancing autophagy with hypoxic preconditioning is an optimizing strategy for EPC therapy of myocardial infarction. Fibrin glue can effectively avoid lose of the transplanted cells and promote the cell survival.

F-1196

IMPROVEMENT OF DYSFUNCTIONAL DIABETIC BONE MARROW-DERIVED ENDOTHELIAL PROGENITOR CELLS BY QUANTITY AND QUALITY CONTROL CULTURE SYSTEM RELATED TO OXIDATIVE STRESS ATTENUATION

Dewi Sukmawati[1], Satoshi Fujimura[2], Sachie Jitsukawa[2], Seigo Itoh[1], Hiroshi Mizuno[2], Takayuki Asahara[3], Hiroyuki Daida[1], Rica Tanaka[2]
[A]Cardiovascular Medicine, [B]Plastic and Reconstructive Surgery, [1]Juntendo University School of Medicine, Tokyo, Japan, [2]Regenerative Medicine Science, Division of Basic Clinical Science, Tokai University School of Medicine, Kanagawa, Japan

Background: Diabetes is associated with reduced vascular repair, as indicated by impaired wound healing and reduced collateral formation in ischemia. This is generally attributed to the adverse effects of hyperglycemia and oxidative stress on vascular biology which lead to dysfunction of the endothelial cells. The homeostasis of vascular repair is accomplished through the contribution of circulating endothelial progenitor cells (EPCs). It has extensively demonstrated that in diabetic patients have less EPCs number and display functional impairment. Our group previously developed and reported the benefit of Quality and Quantity Control (QQc) Culture system in recovering

diabetic EPCs in both function and cell number. However its molecular mechanisms are not yet understood. Our study aim is to investigate the possible underlying mechanism of diabetic EPCs restoration using ex vivo expansion of QQc culture system through modulation of oxidative stress-related mechanism.
Methods: Bone marrow-derived EPCs were harvested from 4 th week diabetic-induced Streptozotocin (DM-EPCs). Cells were sorted for KSL (Lin(-)/c-kit(+)/sca-1(+)) by FACS and expanded 7 days in QQc culture system as a serum free medium containing Stemspan enriched with SCF, thrombopoietin,VEGF, IL-6, and Flt-3 ligand. Oxidative stress marker was measured by ELISA for carbonylated protein (DNPH). Expression of anti-oxidative enzyme genes (MnSOD, glutathione peroxide (GPx), and catalase) were confirmed by RT-PCR. Catalase activity was measured using Amplex Red kit. Annexin V/PI staining was performed for apoptosis detection by FACS. All data were compared in pre and post QQc culture.
Results: Pre QQc DM-EPC showed increase in apoptosis compared to post QQc DM-EPC. After QQc, DM-EPCs demonstrated decrease in apoptosis compared to pre QQc DM-EPCs (8.93% vs 48.1%) respectively. Oxidative stress marker (DNPH) which was high in pre QQc DM-EPCs (1±0.00) were decreased post QQc (0.4±0.10). The relative expression level of anti-oxidative enzymes were low in pre QQc DM-EPCs and were increased in post QQc DM-EPCs as for SOD ((pre-QQc DM-EPCs (3.87 ± 1.1)x10-5) vs post-QQc DM-EPCs ((9.83 ± 5)x 10-5)), GPx (pre QQc DM-EPC (6.12 ± 2.11)x 10-4 vs post QQc DM-EPCs (10.2 ± 4.34)x 10-4) and catalase (pre QQc DM-EPCs (8.05 ± 6.18)x10-5 vs post QQc DM-EPCs (24.4 ± 18.4)x10-5). Catalase activity also demonstrated increase in post QQc DM-EPCs (17.67 ± 3.8 mU/104 cells) compared to pre DM-EPCs (7.74 ± 1.8 mU/104 cells) respectively.
Conclusion: Our results indicate that ex vivo expansion by QQc system improves EPCs function under diabetic condition by activating anti oxidative enzymes and rescuing the cells from oxidative stress. These finding may provide a promising expansion method for functional restoring of diabetic EPCs prior to cells transplantation.

F-1197

ROLE OF FORMYL PEPTIDE RECEPTOR 2 FOR HOMING OF ENDOTHELIAL PROGENITOR CELLS AND ISCHEMIC NEOVASCULOGENESIS

Soon Chul Heo, Yang Woo Kwon, Geun Ok Jeong, Jung Won Yoon, Won Min Mo, Il Ho Jang, Jae Ho Kim
Physiology, School of Medicine, Pusan, Korea, Republic of

Mobilization of endothelial progenitor cells (EPCs) from bone marrow and homing of EPCs to ischemic tissues is required for neovascularization. N-formyl peptides produced by Gram negative bacteria have been implicated in the host defense by stimulating chemotaxis of leukocytes through the mechanisms involving N-formyl peptide receptors. In this present study, we investigated the involvement of the G protein-coupled receptor formyl peptide receptor 2 (FPR2) in the homing of EPCs and the vascular regeneration of ischemic tissues. WKYMVm, an FPR2 agonist, stimulated chemotactic migration and angiogenesis of human EPCs in vitro. Intramuscular injection of WKYMVm attenuated severe hindlimb ischemia and promoted vascular regeneration. When EPCs were transplanted via tail vein into nude mice, they were incorporated into capillary vessels in the ischemic hindlimb, augmented neovascularization, and improved ischemic limb salvage. Intramuscular injection of WKYMVm promoted the homing of transplanted EPCs to the ischemic limb and the vascular regeneration. siRNA-mediated knockdown of FPR2 abrogated WKYMVm-induced chemotactic migration in the tissue culture and the homing of EPCs to the ischemic limb in mice. These results suggest that WKYMVm promotes neovascularization and regeneration of injured tissues by stimulating the homing of EPCs via FPR2-dependent mechanism.

F-1198

IN VIVO AND IN VITRO HIERARCHY OF HUMAN ENDOTHELIAL CELLS ISOLATED FROM PLACENTA AND CORD BLOOD

Jatin Patel[1], Elke Seppanen[1], Mark Chong[2], Julie Yeo[3], Erin Teo[3], Jerry Chan[2], Nicholas M. Fisk[1], Kiarash Khosrotehrani[1]

[1]University of Queensland Centre for Clinical Research, Brisbane, Australia, [2]KK Women's and Children's Hospital, Duke-NUS Graduate Medical School & Yong Loo Lin School of Medicine, National University of Singapore, Singapore, [3]KK Women's and Children's Hospital, Duke-NUS Graduate Medical School & Yong Loo Lin School of Medicine, Singapore, Singapore

The Endothelial Colony Forming Cell (ECFC) assay has been considered the gold standard for establishing the hierarchy among endothelial cells. ECFC are a potential source for cell therapy of various ischemic injuries, due to their high proliferative potential (HPP) and vessel forming capacity. However, ECFC are rare in circulation and thus only nominal amounts can be isolated, even from umbilical cord blood (UCB), limiting their clinical potential. Due to the large vascular capacity of the placenta, we propose greater numbers of ECFC would be present in placental blood vessels compared to UCB and could more readily be isolated and expanded into amounts necessary for clinical application. Based on previous results, we also hypothesized that differential cell surface marker levels could distinguish primitive populations from more differentiated endothelial cells.
ECFC were isolated from matched human term placentas (37-38 weeks) and UCB, obtained with consent after caesarean section. UCB-ECFC was isolated using the well-described ECFC assay, while placental ECFC (PL-ECFC) were isolated via our novel sorting strategy. Positive selection of CD34+ and depletion of CD45+ cells via MACS purification was used to enrich the ECFC population and eliminate contamination with haematopoietic and mesenchymal stem cells. We then demonstrated the CD31 (endothelial marker) expression hierarchy among CD34+CD45- cells. After sorting populations A, B and C, we determined ECFC formation using the ECFC assay. Microarray was compared between PL-ECFC and UCB-ECFC.
Using the ECFC assay we determined that only population 'A' on re-plating led to the formation of characteristic HPP-ECFC. Population 'B' as well as 'C' fractions did not yield any ECFC colonies. On each occasion of plating population 'A' we obtained on average 123 HPP colonies from just 50grams of placental tissue in comparison to UCB (15mL blood) ($p<0.001$), where we obtained an average of 15 HPP colonies. On culture, PL-ECFC manifest cell surface characteristics previously described for UCB-ECFC (CD31+, VEGF-R2+, CD105+, CD144+, CD146+, CD45- and CD73-). Similarly, functional capacity (acetylated-LDL uptake, MatrigelTM tube formation), proliferative potential and in vivo engraftment, vessel formation and reperfusion ability in MatrigelTM, in wounds or in leg ischemia induced by arterial ligation, were observed for PL-ECFC as previously described for UCB. Microarray analysis showed only 33 genes were differentially expressed between PL-ECFC and UCB-ECFC, providing robust evidence that both cells are the same, one from circulation and one a resident vessel cell.
Importantly, upon isolation and culture, the frequency of CD34 expression on PL-ECFC decreased with passaging. CD34Hi cells were situated in the centre of individual colonies. Upon cell sorting and replating, CD34Hi cells provide the continual HPP colonies observed; however this was not observed when CD34Lo and CD34Neg cells were replated. Besides, CD34Hi cells when replated gave rise to all other populations of CD34Lo and CD34Neg.
We report a robust reproducible strategy to isolate ECFC from term placentas based on their cell surface expression, which yields vastly larger quantities of ECFC than UCB. We also demonstrated our PL-ECFC in cell surface expression, genomic profiling and in vivo functional abilities are equivalent to those of UCB-ECFC. We believe PL-ECFC has significant bio-banking and clinical potential due to the large number of cells obtained.

F-1201

MANIPULATION OF MICRORNA CONTENT OF ENDOTHELIAL PROGENITOR CELL-DERIVED EXTRACELLULAR VESICLES

Jasenka Guduric-Fuchs, Anna O'Connor, Reinhold J. Medina, Christina L. O'Neill, Alan Stitt W. Stitt, David Simpson A. Simpson
Centre for Vision and Vascular Science, Queen's University Belfast, Belfast, United Kingdom

Extracellular vesicles (EVs) released from various cell types can mediate paracrine signalling and control important pathophysiological responses. It has become apparent that microRNAs transferred within EVs represent one of the main mechanisms by which they influence the recipient cells. Circulating endothelial progenitor cells (EPCs) release

EVs that are readily taken up by endothelial cells. We hypothesized that EPC derived EVs can be manipulated to deliver specific miRNAs to endothelial cells and thereby modulate their angiogenic response.
Human EPCs isolated from the umbilical cord blood displayed endothelial imunophenotype, being positive for CD31, CD146 and CD105 and negative for haematopoetic markers CD45 and CD14. EVs were collected by ultracentrifugation of the EPC conditioned media. To identify miRNAs exported from EPCs, the small RNA contents of EPCs and their EVs were analyzed by deep sequencing. Several miRNAs were highly enriched in EVs compared to their expression levels in EPCs and there was remarkable consistency in miRNA expression in the cultures from different donors. EVs were then isolated from EPCs transfected with several vectors driving the expression of microRNAs, including miR-146a, and miR-451. Enrichment of these miRNAs in EPC EVs was demonstrated by RT-qPCR.
Following direct transfection of human microvascular endothelilal cells (HMEC) with plasmid vectors driving the expression of miR-146a, downregulation of specific miR-146a predicted target genes such as CCND2, ELAVL1 and CRIM1, was revealed by microarray. When HMECs were incubated with miR-146a enriched EPC derived EVs, these EVs were taken up by HMECs, resulting in the reduced mRNA expression of the same miR-146a targets.
In conclusion, the content of EPC released EVs can be manipulated to include significant amounts of exogenous miRNAs, which can be taken up by endothelial cells. The ability of EV carrying candidate anti- or pro-angiogenic miRNAs to modulate angiogenesis can be assessed in various models and may have therapeutic applications in multiple disease conditions associated with abnormal angiogenesis.

F-1202

MESODERMAL DEVELOPMENT FROM REPROGRAMMED FANCONI ANEMIA CELLS IS AFFECTED BY ALDH2 ENZYMATIC ACTIVITY

Naoya Suzuki[1], Asuka Hira[2], Akira Niwa[1], Keitaro Matsuo[3], Moniru Takata[2], Miharu Yabe[4], Tatsutoshi Nakahata[1], Megumu Saito[1]

[1]Department of Clinical Application, Center for iPS Cell Research and Application, Kyoto university, Kyoto, Japan, [2]Department of Late Effects Studies, Radiation Biology Center, Kyoto university, Kyoto, Japan, [3]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Aichi, Japan, [4]Department of Cell Transplantation, Tokai University Hospital, Kanagawa, Japan

[Introduction]
Fanconi anemia (FA) is a genome instability disorder with clinical characteristics including progressive bone marrow failure (BMF), developmental abnormalities, and increased occurrence of leukemia and cancer. To date 15 genes have been implicated in FA, and their products form a common DNA repair network often referred to as “FA pathway”.
Since cells derived from FA patients are hypersensitive to treatments that induce DNA interstrand cross-links (ICLs), the FA pathway has been considered to function in ICL repair. However, it still remains unclear what type of endogenous DNA damage is repaired through the FA pathway and is the cause of phenotypes in FA patients. Recent studies have suggested that cells deficient in the FA pathway are also sensitive to formaldehyde and acetaldehyde. Aldehydes may create DNA adducts including ICLs or protein DNA crosslinking. These results raise a possibility that the FA pathway prevents BMF by mitigating genotoxicity due to endogenous aldehydes. Since ALDH2 deficiency is prevalent in East Asian populations, Japanese FA patients deficient in ALDH2 enzymatic activity provide a cell source which enables test the role of ALDH2 and aldehyde metabolism in human FA patients.
[Results and discussion]
In FA fetus, p53/p21 axis has already activated in fetal liver (Ceccaldi, Cell stem cell, 2012), indicating the possibility that hematopoietic defects in FA patients originates from an earlier developmental stage. Since human hematopoietic system originates from embryonic mesoderm, we set out to estimate the role of ALDH2 and FANCA pathway during early embryogenesis. For this, we reprogrammed somatic cells from a patient with ALDH2 GA genotype and observed their in vitro mesodermal differentiation. We first introduced reprogramming factors into fibroblasts by episomal vectors, and obtained colonies which are morphologically compatible with human induced pluripotent stem cells (iPSCs). We obtained gene-completed FA-iPSCs (designated as cFA-iPSCs) for control study.

To evaluate the impact of ALDH2 activity on iPSC- or iPSC-derived mesodermal differentiation, we next adapted the previously reported serum-free monolayer culture system (Niwa et al., PlosONE, 2011). Both FA- and cFA-iPSCs showed similar differentiation manners with conventional embryonic stem cells and iPSCs, and percentages of KDR+ mesodermal progenitors including KDR+CD34+ common hemoangiogenic progenitors were comparable. Notably, ALDH2 agonist Alda1 (ref: PMID:18787169) did increase only FA-iPSC-derived mesodermal progenitors but not cFA-iPSCs. These data supported the hypothesis that mesodermal development towards hematopoietic cells in human can be affected by ALDH2 activity in the absence of FA pathway.
To confirm the hypothesis, next we set out to assess whether the variation in ALDH2 affects symptoms in Japanese FA patients. Strikingly, we found that progression of BMF was strongly accelerated in patients deficient in ALDH2 activity compared to those with wild type ALDH2. This result indicates the possibility that aldehydes affect in early hematopoietic development in FA patients.
Overall, our results from FA-iPSCs and clinical study indicate that the variation in ALDH2 affects the occurrence of bone marrow failure in FA patients, and that hematopoietic defect in FA patients is caused by aldehydes in early mesodermal developmental stage.

F-1203

HEMOGENIC ENDOTHELIAL CELLS SUPPORT THE FORMATION OF HETEROGENEOUS INTRA-AORTIC HEMATOPOIETIC CLUSTERS

Joan P. Zape, Ann C. Zovein
University of California San Francisco, San Francisco, CA, United States

Recent data suggest that hematopoietic stem cells (HSCs) arise during a short time window in development from specialized endothelial cells collectively termed as hemogenic endothelium (HE). The precise mechanism of HSC generation from hemogenic vascular sites is currently unknown. The dorsal aorta (DA) is known to harbor hemogenic endothelial cells typified by the presence of intra-aortic hematopoietic cell clusters (IAHCC). We adapted and optimized murine explant culture assays of the dorsal aorta and the surrounding region known as the aorta-gonado-mesonephros (AGM) region. By time-lapse microscopy, we examined live 200um cross-sections of wild type murine dorsal aortas. Our data suggest that the initiation of the IAHCCs occurs in the endothelial layer. Additionally, we observed two or more distinct hemogenic endothelial cells that contact each other and interact during early IAHCC formation, thus suggesting a non-clonal origin for IAHCCs. When we examined the early 3-4 cell IAHCCs by immunofluorescence, we find that among these cells, there exists a differential expression pattern of the mitotic marker Ki67 and endothelial adhesion marker CD31. Furthermore, live-imaging of labeled mouse lines that allow evaluation of the endothelium and cell proliferation reveal dynamic behavior among the differentially labeled and unlabeled cells. The data suggest so far that there are at least two cell subtypes that comprise IAHCC with distinct expression pattern and functional properties. The morphological characteristics of the cells in the early IAHCCs and their interactions resemble a de novo niche. This niche formation occurs prior to the observed expansion of cell number in the IAHCC. Overall, our data suggest that hemogenic endothelial cells contribute to and support the formation of intra-aortic hematopoietic clusters that function as a transitory niche for HSC specification during early embryonic development.

F-1204

THERAPEUTIC POTENTIAL OF HUMAN-INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS IN A BLEOMYCIN-INDUCED SCLERODERMA MOUSE MODEL

Manizheh Azhdari
Researcher (MS.c), Tehran, Iran, Islamic Republic of

Vascular injury and destruction of endothelial cells are the early events in scleroderma (SSc) patients. This study aims to investigate the therapeutic potential of human-induced pluripotent stem cell-derived ECs (hiPSC-ECs) to treat SSc. We have assessed the functional differentiation of hiPSC-ECs and compared them with human embryon-

ic stem cell-derived ECs (hESC-ECs) by a variety of in vitro experimental approaches. Additionally, we evaluated the therapeutic potential of hiPSC-ECs in a bleomycin-induced SSc mouse model. Our results demonstrated that hiPSC-ECs and hESC-ECs showed similar maximum expressions of FLK1 (early EC marker) at day five during differentiation. The hESC-ECs and hiPSC-ECs also expressed late EC markers CD31 (68% and 75%), CD144 (50% and 61%), CD146 (46% and 61%), and DiI-ac-LDL uptake (55% and 63%), respectively. Analyses of the transplantation of sorted CD31-positive hiPSC-ECs into the bleomycin-induced SSc mouse model demonstrated that these cells participate in recovery of the damaged vessels. There was a reduction in collagen content; the number of total and degranulated mast cells returned to their normal state, and bleomycin-induced wounds as well as skin fibrosis improved four weeks after transplantation of hiPSC-ECs. Additionally, this is the first study to determine the therapeutic potential of vascular cells from hiPSCs in the treatment of an SSc model. In the future, these may be used as an appropriate source for the treatment of SSc patients.

Other

F-2011

CONSERVING ENDANGERED SPECIES WITH INDUCED PLURIPOTENT STEM CELLS

Susanne C. Montague[1], Inbar Friedrich Ben-Nun[1], Marlys L. Houck[2], Suellen J. Charter[2], Yu-Chieh Wang[1], Suzanne E. Peterson[1], Louise C. Laurent[3], Oliver A. Ryder[2], Jeanne F. Loring[1]

[1]Chemical Physiology, The Scripps Research Institute, La Jolla, CA, United States, [2]The San Diego Zoo Institute for Conservation Research, Escondido, CA, United States, [3]Reproductive Medicine, University of California San Diego, San Diego, CA, United States

Unique plant and animal species are disappearing from the earth at an alarming rate due to a number of factors including habitat loss, climate change and poaching. One approach to stop the loss of these species has been captive breeding programs in zoos and other wild life preserves. While this has been successful for some species, it has not worked for others. A new potential solution to help stave off this loss of species is the use of induced pluripotent stem cells (iPSCs) to generate new individuals of the species. iPSCs can divide indefinitely in culture and can be differentiated into any cell type in the body. Importantly, it was recently shown that iPSCs can be differentiated into functional eggs and sperm. Thus, using iPSCs, it may be possible to generate new individuals of a particular species. For this project, we made use of the Frozen Zoo at the San Diego Zoo's Institute for Conservation Research, which is an extensive collection of cryopreserved primary fibroblast cultures, collected from over 8600 individual vertebrates from approximately 800 species. We used viral-based vectors to reprogram fibroblasts from a variety of different endangered species including the drill, Mandrillus leucophaeus, and the northern white rhinoceros, Ceratotherium simum cottoni. During the course of these experiments we determined that Moloney murine leukemia virus-based retroviral vectors could effectively deliver the reprogramming factors into the northern white rhinoceros fibroblasts when pseudotyped with vesicular stomatitis virus G envelope protein (VSV-G), but not with the amphotrophic envelope protein. The VSV-G pseudotyped retroviral vectors also efficiently transduced the drill fibroblasts. We were able to reprogram both the rhinoceros and drill fibroblasts using the human OCT4/POU5F1, SOX2, KLF4, and MYC cDNA sequences, suggesting that retroviral vectors carrying human reprogramming factor sequences and pseudotyped with VSV-G may be widely applicable for generating iPSCs from a variety of species. We are currently using the same methods to reprogram fibroblasts from the Javan Banteng, Somali Wild Ass and Black Footed Cat.

F-2012

IDENTIFICATION OF HUMAN NEPHRON STEM/PROGENITOR CELLS THAT CAN GENERATE RENAL STRUCTURES AND HALT THE PROGRESSION OF CHRONIC RENAL DISEASE

Orit Harari-Steinberg[1], Sally Metsuyanim[2], Dorit Omer[1], Yehudit Gnatek[1], Ella Buzhor[3], Oren Pleniceanu[1], Sara Pri-Chen[1], Rotem Gershon[1], Herzel Ben-Hur[4], Bruce J. Aronow[5], Ronald S. Goldstein[2], Benjamin Dekel[6]

[1]The Pediatric Stem Cell Research Institute, Edmond & Lily Safra Children's Hospital, Sheba Medical Center, Tel-Ashomer, Israel, [2]Mina and Everard Goodman Faculty of Life Sciences, Bar-IlanUniversity, Ramat-Gan, Israel, [3]Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel, [4]Department of Obstet. & Gynecology, Assaf Harofeh Hospital, Assaf Harofeh, Israel, [5]Division of Molecular and Developmental Biology, Department of Pediatrics, University of Cincinnati, Childrens Hospital Medical Center,, Cincinnati, OH, United States, [6]Pediatric Stem Cell Research Institute, Center for Regenerative Medicine, Sackler School of Medic, Sheba Medical Center, Tel Aviv University, Tel-Ashomer, Tel Aviv, Israel

Identification of tissue-specific renal stem/progenitor cells with nephrogenic potential is a critical step in developing cell-based therapies for renal disease. Neither haematopoietic nor mesenchymal stem cells, the most accessible human stem cells, can be used to derive true renal progenitors. In the human kidney, stem/progenitor cells are induced into the nephrogenic pathway to form nephrons until 34th week of gestation, and no equivalent cell types can be traced in the adult kidney. Nevertheless, committed human nephron progenitor cells (hNPCs) have yet to be isolated during this nephrogenic window of opportunity. Here we show that growth of human fetal kidneys in serum-free defined conditions selects for epithelial lineage and that prospective isolation of hNPCs by NCAM1 immunosorting identifies a mitotically active population with *in vitro* clonogenic and progenitor properties. After transplantation onto the chorio-allantoic membrane of the chick embryo, these cells -but not differentiated counterparts - efficiently formed proximal, loop of henle and distal nephron tubules. Moreover, hNPCs engrafted and integrated in diseased murine kidneys and treatment of renal failure in the 5/6 nephrectomy kidney injury model with repeated hNPC injection had beneficial effects on renal function halting disease progression. These findings constitute the first definition of an intrinsic nephron precursor population derived from human fetal kidney, with major potential for cell-based therapeutic strategies.

F-2013

TREATMENT OF VENOUS LEG ULCERS WITH BONE MARROW DERIVED STEM CELLS: A PILOT STUDY

Cristina L. Touriño[1], Gabriela Otero[2], Caroline Agorio[2], Ana Tchekmedyian[5], Sujanov Alexandra[1], Andrés Rodríguez[3], Lourdes Echarte[1], Daniel Leal[1], Monica Montelongo[3], Begoña Valdes[2], Ismael Rodríguez[3], Lilian Diaz[4]
[A]Area de Terapia Celular y Medicina Regenerativa. Dept. Básico de Medicina, [B]Departamento de Dermatología, [C]Departamento de Hemoterapia y Medicina Transfusional, [D]Departamento de Hematología, [1]Hospital de Clínicas Dr. M. Quintela. Universidad de la República, Montevideo, Uruguay, [2]CETEP, Centro Hospitalario Pereira Rossell. MSP, Montevideo, Uruguay

Leg ulcers have a prevalence of 1-3% in European countries and the most common cause of leg ulceration is venous insufficiency. Typically leg ulceration is a chronic recurrent condition with considerable cost both to the patient and to the health services. Chronic wound healing is a time consuming process that occasionally is refractory to standard therapy of moist healing. This fact makes chronic wounds candidate to new strategies in the emergent field of regenerative medicine, with the aim to stimulate missing or dysfunctional components of wound healing process. In recent years, different growth factors like human granulocyte-macrophage-colony stimulating factor (GM-CSF) or platelet-derived growth factor (PDGF) has been employed to treat leg ulcers from different etiology. Moreover, different cells types (autologous epidermal cell, blood mononuclear cells, bone marrow derived cells and mesenchymal stem cells) have been used to treat different wounds that showed improvement in healing rates.
OBJECTIVE. A pilot study was conducted to determine the safety, feasibility and efficacy of cell therapy with bone marrow derived stem cells (BMDSC) as a complementary therapy for chronic venous leg ulcers.
METHODS. This study was approved by the INDT Ethics Committee from Uruguay and was conducted after obtaining appropriate informed consent. We have enrolled four patients, two male and two female with ranging age from 59 to 72 years, from the Clinic of Dermatology at the Hospital de Clinicas of Uruguay. A total of six venous legs ulcers with a time evolution of ten years or more were included. All patients received standard treatment with moist healing and compression for six month before enrollment. Patient with acute wounds, diabetes mellitus, ulcers of non

venous etiology were excluded. Bone marrow was aspirated from bilateral iliac crests centrifuged and processed under sterile condition in laminar flow. Multiple injections of 0.2 ml each were made, separated by a distance of 2 cm from each other covering the edge and the bed of the ulcers.
RESULTS. Three days after the procedure the patients were evaluated. No hematoma, bleeding, eczema, fibrin or hypergranulation were observed in the wound bed. No complication was observed at the puncture site and only one patient referred transient pain. The volumes injected varies from 23,4 to 4,2 ml with a mean concentration of 6.7 (SD 8.5) x 107 nucleated cells per ml and a mean concentration of 788 (SD865) CD34 cells per ul. The mean area decreased from 495,75 cm2 (SD: 519,21, Min 30 cm2 and Max 1238 cm2) prior treatment to 395,45 cm2 (SD: 466.03, Min 3,81 cm2 Max 1071 cm2) at two month and 281,53 cm2 (SD: 413 Min 2,13 Max 895) at twelve month after the procedure. In addition, one ulcer healed within the first month and leg ulcer´s pain decreased in all patients.
CONCLUSIONS. These results suggest that autologous BMDSC transplantation can lead to an improvement in wound healing rates and seem to be a safe complementary treatment. However, chronic ulcers with big area don´t achieve total closure so additional injections or combination of treatments will be required. Proper control studies need to be performed.

F-2014

IMPLICATING THE PLURIPOTENCY FACTOR LIN28 IN KIDNEY DEVELOPMENT AND WILMS TUMOR

Achia Urbach[1], Yermalovich Alena[1], Kaite Spina[1], Shinoda Gen[1], Hao Zho[1], Benjamin Dekel[2], George Q. Daley[1]
[1]Stem Cell Transplantation Program, Division of Pediatric Hematology/Oncology, Boston Children's Hospital, Boston, MA, United States, [2]Pediatric Stem Cell Research Institute, Sheba Medical Center, Sackler School of Medicine, Tel Aviv, Israel

Lin28, first recognized as a heterochronic gene in C. elegans, is a highly conserved RNA binding protein that modifies gene expression via direct binding of mRNAs and by blocking the processing of the let-7 family of tumor suppressor microRNAs. In vertebrates, Lin28a and its paralog Lin28b are highly expressed in stem and progenitor cells of the early embryo, and play important roles in the balance of self-renewal, proliferation, and differentiation. Lin28 is also one of the "reprogramming factors" that can reprogram adult cells into induced pluripotent stem cells. Overexpression of Lin28 has been detected in a wide array of malignancies, including colon, breast, kidney, liver, leukemia, and several pediatric embryonal tumors. To study whether Lin28 over-expression might predispose to tumorigenesis in murine models, we generated transgenic strains that over-express Lin28a constitutively in all tissues. General Lin28a over-expression produced perinatal lethality. At necropsy we found enlarged kidneys, and histologic assessments of prenatal embryonic kidneys harvested at E18.5 showed developmental immaturity and reduced differentiation in the nephrogenic zones of transgenic kidneys relative to controls. We transplanted E18.5 transgenic and control kidneys under the kidney capsule of immunodeficient mice, and observed tumor formation in 70% (7/10) of the recipients of Lin28a transgenic kidneys but none in control (0/9). Histologic and immunohistochemical analysis of the tumors with markers of early kidney lineages revealed a complex mixture of undifferentiated cells and mature differentiated tubules, reminiscent of the nephrogenic rests and blastema formation of Wilms Tumor, the most common pediatric tumor of the kidney. During normal mouse kidney development, Lin28a is highly expressed in the early metanephric mesenchyme at E11.5, then persists until E13.5 at lower levels in the cap mesenchyme cells, which represent the precursor population for the formation of nephrons, the functional unit of the kidney. Cap mesenchyme cells normally proliferate until the second postnatal day, after which they undergo synchronized differentiation to determine the final number of nephrons that will persist lifelong in the adult. Transgenic overexpression of Lin28a resulted in persistent high level expression of Lin28a in cap mesenchyme, which delayed and compromised this wave of differentiation, sustaining persistent nephrogenesis and ultimately resulting in tumor formation. We examined 15 cases of human Wilms Tumor and found prominent overexpression of the LIN28B paralog in a third of cases. Previously, we had identified activation of LIN28B expression in two cases of human Wilms Tumor associated with translocation of the LIN28B locus on chromosome 6 (Viswanathan et al, Nat Genetics, 2009). Understanding the mechanism by which Lin28 contributes to the developmental maturation of

early kidney precursor populations will inform our understanding of normal kidney development and Wilms Tumor formation. Moreover, the capacity for Lin28 to drive nephrogenesis raises the possibility that controlled reactivation of Lin28 in the adult kidney might represent a new strategy for kidney repair and regeneration.

F-2015

MESENCHYMAL STEM CELLS STIMULATE AN IMMUNE RESPONSE BY PROVIDING IMMUNE CELLS WITH TOLL-LIKE RECEPTOR 2 LIGAND

Ada Weinstock, Meirav Pevsner-Fischer, Dov Zipori
Molecular cell biology, Weizmann Institute of Science, Rehovot, Israel

Objective: Investigating the effect of TLR2 ligand on the immunosuppressive function of MSCs
We have previously published that mesenchymal stromal cells (MSCs) express toll-like receptor (TLR) 2 and are activated by its ligand Pam3Cys. TLR2 is an important component of the innate immune system, as it recognizes bacterial lipopeptides and thus activates lymphocytes. In line with other studies on the immunosuppresvie nature of MSCs, our research shows that MSCs inhibit interferon (IFNγ) production by splenocytes in vitro. However, when treated with Pam3Cys, both wild type (WT) and TLR2 KO MSCs induce IFNγ production by WT splenocytes. To investigate the fate of Pam3Cys in MSCs, we treated MSCs with rhodamine or biotin conjugated Pam3Cys. We show that Pam3Cys binds similarly to WT and TLR2 KO MSCs, indicating that the binding is independent of TLR2. Because Pam3Cys is a lipopeptide, we hypothesized that its fatty acid residues adsorbs to the MSC membrane. Using Pam3Cys that lacks fatty acid residues we demonstrated that indeed the fatty acids are responsible for the adsorption of Pam3Cys to MSCs. In vitro, MSCs adsorb Pam3Cys and subsequently secrete it, while maintaining the ligands ability to activate splenocytes. In vivo, MSCs treated with Pam3Cys and administered systemically, transfer the Pam3Cys to immune cells of the peripheral blood and induce secretion of pro-inflammatory cytokines. Moreover, MSCs loaded with Pam3Cys and injected intra foot pad promote a pro-inflammatory immune response in the popliteal lymph nodes. Overall, we demonstrated that MSCs adsorb Pam3Cys and subsequently release it, to activate an immune response, both in vitro and in vivo.

F-2016

TGFβ REVERSES THE IMMUNOSUPPRESSION OF MESENCHYMAL STEM CELLS BY REDUCING iNOS EXPRESSION

Chunliang Xu, Yufang Shi
Chinese Academy of Sciences, Institute of Health Sciences, Shanghai Institute for Biological Sciences, Shanghai, China

Mesenchymal stem cells (MSCs) possess potent immunosuppression capacity and have been shown to provide therapeutic effects in many autoimmune disease models. But the mechanism of immunosuppression of MSCs is still not clear. Our previous data showed that mouse MSCs inhibit T cells proliferation in an iNOS-dependent manner both in vitro and in vivo. Here, we report that TGFβ reversed the immunosuppression of MSCs when added into the MSCs and T cells co-culture system. Using TGFβ unresponsive MSCs, we demonstrated that the target cell of TGFβ is MSCs. Further studies showed that TGFβ decreased the nitrate concentration in the co-culture systems, and the expression of iNOS induced by inflammatory cytokines in MSCs. The inhibition was at transcription level and in a SMAD3-dependent manner. Interestingly, MSC-derived TGFβ acted in autocrine manner to reduce iNOS expression from itself. Lastly, we found that TGFβ-unresponsive MSCs have enhanced immunosuppressive capability compared with normal MSCs. In summary, our study revealed a novel role of TGFβ in the immunosuppression of MSCs.

F-2017

MESENCHYMAL STEM CELLS PREVENT PHYSICAL STRESS INDUCED LYMPHOCYTE APOPTOSIS THROUGH IL4

Gang Cao, Zhang Siyu, Yang Qian, Xu Chunliang, Ying Wang, Yufang Shi

Institute of Health Science, Shanghai, China

Chronic stress, through the neuro-endocrine axis, has dramatic impact on the immune system and contributes significantly to the onset and progression of a variety of diseases, such as tumor, autoimmunity, and infection. However, few countermeasures have been developed to alleviate the deleterious effects of stress on the immune system. Recent studies in animals and humans have demonstrated that mesenchymal stem cells (MSCs) could treat various immune related diseases and promote general health. In the present study, we employed bone marrow-derived MSCs and demonstrated that in vivo administration of these cells could prevent stress-induced lymphocyte apoptosis and subsequent lymphocyte number reduction in mice subjected to restraint stress. Although it has been shown that elevation of glucocorticoid in circulation is responsible for such stress-induced lymphocyte reduction, we did not find any significant effect of MSCs on the serum level of glucocorticoid, indicating that the effect of MSCs is exerted by affecting glucocorticoid-induced apoptosis. We therefore tested the effect of various cytokines on dexamethasone-induced lymphocyte apoptosis. Among all cytokines tested, we found that IL-4 exhibited strongest protection. Interestingly, when neutralizing antibody against IL-4 was administered to MSC-treated restraint mice, the protective effect of MSCs was eliminated. Furthermore, in mice deficient in STAT6, a key molecule in IL-4 receptor-mediated signaling, MSCs did not show any protection against restraint stress-induced lymphocyte reduction. Taken together, our study revealed that MSCs could effectively prevent stress induced lymphocyte apoptosis in an IL-4 dependent manner and provided important information for the development of countermeasures for chronic stress.

F-2018

DIFFERENTIAL MICRORNA SIGNATURE OF HUMAN MESENCHYMAL STEM CELLS FROM DIFFERENT SOURCES REVEALS AN “ENVIRONMENTAL-NICHE MEMORY” FOR BONE MARROW STEM CELLS

Enrico Ragni, Tiziana Montemurro, Elisa Montelatici, Cristiana Lavazza, Mariele Viganò, Valentina Parazzi, Valentina Boldrin, Mario Barilani, Paolo Rebulla, Rosaria Giordano, Lorenza Lazzari
Cell Factory - Department of Regenerative Medicine, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy

Human mesenchymal stem cells (MSCs) are multipotent cells offering valuable hopes for the treatment of degenerative diseases. MSCs can be found among differentiated cells in many tissues and organs but, unfortunately, their phenotypic similarity hinders a robust cell characterization and discrimination from diverse tissue harvests. MicroRNAs (miRNAs) are crucial managers of gene expression with intriguing and still poorly known roles in stem cell maintenance and differentiation. To identify miRNAs that can discriminate among MSCs, we performed a whole-genome comparative miRNA expression profiling analysis on adipose (AD), bone marrow (BM) and cord blood (CB) derived MSCs, all three considered among the most promising in the field of regenerative medicine. miRNA expression patterns were very similar, meeting their extensive phenotypic and functional overlaps. An in depth comparison of the few most differentially expressed miRNAs allowed the identification of a highly restricted molecular signature consisting of 5 BMMSC, 11 ADMSC and 11 CBMSC specific miRNAs. Functional analysis of their validated targets allowed the identification of an “environmental-niche memory” for BMMSC and an “epithelial” commitment for ADMSC, providing new insights into the molecular mechanisms discriminating between these MSCs, a crucial element to identify the most appropriate stem cell source for clinical application.

F-2021

POTENTIAL MARKER GENES ASSOCIATED WITH OSTEOGENIC PROPERTIES OF DENTAL FOLLICLE STEM CELLS (DFSCS).

Maryam Rezai Rad, Chunhong Li, Gary E. Wise, Shaomian Yao
Louisiana state university, Department of Comparative biomedical sciences, Baton Rouge, LA, United States

Background and Objectives: Dental follicle stem cells (DFSCs) have been shown to possess a strong osteogenic capability, which makes them a valuable source for bone regeneration. Such osteogenic capability is gradually reduced during in vitro culture or expansion. This study is aimed to elucidate the potential marker genes associated with the osteogenic properties of DFSCs, and to determine what genes are associated with the reduction of osteogenic capability in long-term cultures of DFSCs.
Methods: DFSCs and their non-stem cell counterparts dental follicle cells (DFC) were isolated from dental follicles of rat pups. Expression of 42 common stem cell markers in the DFSCs and DFC was evaluated using real-time RT-PCR and Western blotting. The genes that were expressed significantly higher in DFSCs than in the DFC were selected for further analysis of their expression in different passages of DFSCs with real-time RT-PCR. Based on this analysis, genes whose expression associated with reduction of osteogenic capability were identified, and expression of these genes was knocked down by siRNA to determine which genes may be involved in regulating the osteogenic capability of DFSCs.
Results: Fifteen of the 42 marker genes were expressed 2-fold or greater in DFSCs than in DFC. Of them, Musashi (RNA-binding protein Musashi homolog 1), C-Kit (tyrosine-protein kinase Kit), Notch-1(Notch homolog 1) and DMP1 (Dentin matrix protein 1) had an average of 33.8, 43.03, 57.79 and 856 fold increase respectively, in the DFSCs when compared with the DFC. This increase at the translational level was confirmed by Western blotting. These genes also showed decreased expression with progression of passaging of the DFSCs. Knockdown of Musashi and DMP1 expression in the early passage DFSCs resulted in reduction of osteogenesis. However, knockdown of Notch-1 and C-Kit did not affect osteogenesis of DFSCs.
Conclusions: Expression of Musashi, C-Kit, Notch-1 and DMP1 was significantly greater in the DFSCs than in non-stem cell DFC. Reduction in expression of these markers was associated with the reduction of osteogenic capability of the DFSCs in later passages., Thus, these genes might be used as markers for loss of osteogenic properties after long-term culture of DFSCs. Together with our gene knockdown experiments, we conclude that these genes (Musashi and DMP1) may function to regulate osteogenesis in DFSCs.

F-2022

TOPOISOMERASE I INHIBITOR, CAMPTOTHECIN, INDUCES APOPTOSIS OF HUMAN EMBRYONIC STEM CELLS

Carolina P. García, Guillermo A. Videla Richardson, Nicolas A. Dimopoulos, Diego A. Riva, Damian D. Fernandez Espinosa, Leonardo Romorini, Santiago G. Miriuka, Gustavo E. Sevlever, **Maria Elida Scassa**
FLENI, Buenos Aires, Argentina

Human embryonic stem cells (hESC) possess two unique properties that distinguish them from many other cell types: the ability to self-renew indefinitely in culture under permissive conditions and the capability of giving rise to all cell types of embryonic lineage under the guidance of appropriate developmental cues (pluripotency). Maintaining genomic integrity is vital for stem cells and their function as primary progenitors, since mutations will severely compromise all derived cell lineages. Currently, the mechanisms that protect the genome in rapidly proliferating hESCs are minimally understood. The pathways controlled by the ataxia telangiectasia-mutated (ATM) and ATM-related (ATR) proteins represent one of the main pathways by which cells react to DNA damage in somatic cells. In a previous study, we determined that the ATM checkpoint signaling cascade is intact in WA09 hESCs. Here, we investigated how WA09 hESC line reacts to replication mediated double-strand DNA breaks triggered by the topoisomerase I inhibitor, camptothecin (CPT), and whether this genomic insult evokes DNA repair pathways and/or cell death. Using immunofluorescence microscopy we found that hESCs respond to DNA damage by rapidly inducing caspase-3 activation and PARP-1 cleavage concomitantly with phospho-H2AX foci formation. Western blot analysis revealed that p53 phosphorylation on serine 15 occurs within 3 h after CPT addition. Although a marked and sustained increase in $p21^{WAF1}$ transcripts was observed by qPCR (5 fold) upon damage, $p21^{WAF1}$ protein levels were undetectable at all tested time points. We also found that the majority of hESCs were undergoing cell death via caspase-related apoptosis following induction of DNA damage. During the cellular DNA damage response, p53 can either promote cell survival by activating cell cycle arrest

and DNA repair to maintain genomic integrity or direct cells to undergo apoptosis to eliminate extensively damaged cells. Our data suggest
that following CPT treatment, hESCs initiate a p53-dependent programmed cell
death to ensure that its genomic integrity will not be compromised. The absence of $p21^{WAF1}$ protein to sustain cell cycle arrest
supports this premise.

F-2023

ADMINISTRATION OF FETAL KIDNEY STEM CELLS ATTENUATES PROGRESSION OF ISCHEMIA/REPERFUSION INDUCED ACUTE RENAL FAILURE IN RATS

Sachin H. Jadhav, Ashwani K. Gupta, Garikipati VN Srikanth, Soniya Nityanand
Stem Cell Research Facility, Department of Hematology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India

Tissue-specific stem cells represent an excellent cell-type for regenerative therapy of highly complex organs like kidney. Few attempts have been made to characterize and use specific progenitor/stem cell populations from the developing kidney. However, lack of definitive surface markers on stem/progenitor cells in the kidney have hampered the identification of these cells in developing kidneys. Moreover, the mechanisms underlying reno-protective effects of these cells particularly in acute renal failure (ARF) remain obscure. In the present study, we isolated and characterized a stem/progenitor cell population from developing rat kidneys and investigated their therapeutic potential in a rat model of ischemia/reperfusion (I/R) induced ARF.
We isolated and cultured fetal kidney stem cells (fKSC) from Sprague Dawley (SD) rat fetuses at gestation day 16. fKSC between passages 3 to 5 were characterized by comprehensive flow cytometery, immunocytochemistry and RT-PCR analysis and their differentiation potential was evaluated. To test the therapeutic efficacy of fKSC, I/R induced rat model of ARF was developed by clamping both renal pedicels for 45 min. 24 h after reperfusion, animals were divided in 2 groups viz. saline treated (n=6) and fKSC treated (n=6) groups. PKH26 labeled fKSC (2 x 106 cells per animal) or saline alone (150 µl each) was injected through tail vein. Blood was collected at 24, 48, 72, and 96 h after reperfusion to assess kidney function. The animals were sacrificed 96 h after reperfusion and evaluated for homing of injected cells to damaged kidneys, attenuation of ARF progression, and expression of growth factors-,pro/anti-inflammatory cytokines- and pro/anti-apoptotic genes in the kidney tissues.
Immunophenotypic analysis of fKSC showed that these cells were positive for mesenchymal and embryonal markers. The fKSC also expressed a variety of renal stem/progenitor cell markers as revealed by RT-PCR analysis. They exhibited potential to differentiate into cells of all the three germ layers. When injected 24 h after reperfusion, PKH26 labeled fKSC were detected in tubules and glomeruli of damaged kidney. There was a significant improvement in both degree of tissue injury and kidney functions as evidenced by rapid resolution of tubular structural damage, tubular cell proliferation, decrease apoptosis of tubular epithelial cells and normalization of blood urea nitrogen (BUN), creatinine and neutrophil gelatinase-associated lipocalin (NGAL) levels. Our data suggested that these beneficial effects were predominantly mediated by paracrine and immunomodulatory factors. We therefore concluded that fKSC could be a well-suited stem cell type for the treatment of potentially devastating and largely treatment resistant clinical ARF. However, it would be critical to investigate the therapeutic efficacy of this approach in different pre-clinical models of ARF.

F-2024

NEW FUNCTION AND APPLICATION OF XYLOSE AS A CELL CULTURE SOLUTION.

Sakiko Takizawa-Shirasawa[1], Akito Matsushima[1], Tadayuki Yokoyama[1], Daiki Fujita[2], Fengming Yue[2], Ken Matsumoto[3], Daihachiro Tomotsune[2], Akiko Yoshitome[2], Yuriko Suzuki[1], Katsunori Sasaki[2]
[1]Bourbon Institutes of Health, Matsumoto, Japan, [2]Histology & Embryology, Shinshu University School of Medicine, Matsumoto, Japan, [3]Nissui Pharmaceutical Co.LTD, Tokyo, Japan

D-xylose, monosaccharide containing five carbon atoms, is not metabolized in the human body and almost excreted into a urine, and partly converted to D-threitol in man, however it is known to be absorbed from the small intestine. We previously reported that novel functions of xylose. As for mouse embryonic stem cells (mESCs), undifferentiated state was maintained and proliferation was suppressed in the present of xylose (ISSCR 10th Annual Meeting). In the present study, we investigated that influence on other cells, such as HepG2 (human liver tumor cell line), MCF7 (human breast tumor cell line) and Panc1 (human pancreatic tumor cell line), primary hepatocyte and human iPS cell-derived neuronal cells. Interestingly, sensibility of xylose to each cells was different. All kinds of tested cells except for HepG2 were maintained slow proliferated in Xylose, and re-proliferated normally when changing culture medium back into Glucose which was including culture medium commonly. However, HepG2 was not survived in Xylose.
Though we found a novel nature of xylose, the mechanism of xylose to maintain slow proliferation state remained to be understood. We performed comprehensive analysis of mESCs by DNA microarray to investigate how xylose worked in the cells. Pathway analysis from microarray showed cell cycle regulatory genes from mESCs culture in xylose for 8 days that control G1 checkpoints and passage from G1 to S phase, such as Ccnd, Cdk and Ccne were down-regulated compared with mESCs were maintained at undifferentiated status in the present of glucose for 2 days. In addition, principal oxidative stress response genes, such as MapK10, Gpx and Sod, involved in stress-regulated MAP kinase signaling pathway were down-regulated. These findings suggested the ability of xylose to avoid the cells from explosive proliferation and oxidative stress. The new application of Xylose in cell biology, especially for ES cell research might be raised.

Technologies for Stem Cell Research

F-2027

DETECTION OF CHANGES IN OCT4 mRNA LEVEL IN EMBRYONIC STEM CELLS UNDERGOING DIFFERENTIATION USING A NOVEL LIVE CELL RNA DETECTION TECHNOLOGY

Kevin Su, Don Weldon, Grace Johnston, Matthew Hsu, **Lucas Armstrong**
EMD Millipore, Temecula, CA, United States

Although cell surface protein markers for embryonic stem cells have been widely employed for detecting and sorting pluripotent and differentiated stem cell populations, the use of RNA markers in live cells detection and sorting has been much more challenging. A method for detecting specific RNAs in live stem cells would be very beneficial for identifying and separating subpopulations of stem cells and their progeny for which cell surface markers are lacking. Here we describe a detection method that permits visualization of RNAs in live cells, without the need for transfection reagents. The detection technique employs gold nanoparticles, functionalized with fluorescently tagged oligonucleotides, that enter the cell by means of the cell's native endocytosis apparatus. Upon binding of the target RNA sequence, a fluorescent signal (flare) is released, thereby rendering the cell to be detected by any fluorescence analysis platform. We employed this technique to detect changes in Oct4 mRNA in mouse embryonic stem cells induced to differentiate by treatment with retinoic acid, and thus were able to demonstrate the down-regulation of Oct4 mRNA in the differentiated cells. Detecting RNA expression levels in live stem cells, with the capability for utilizing the same cells in downstream applications, enables researchers to perform analyses and methods with intracellular RNAs which were previously thought to be impossible.

F-2028

A TALEN GENOME-EDITING SYSTEM FOR GENERATING HUMAN STEM CELL-BASED DISEASE MODELS

Chad A. Cowan
Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States

Transcription activator-like effector nucleases (TALENs) are a new class of engineered nucleases that are easier to design to cleave at desired sites in a genome than previous types of nucleases. We report here the use of TALENs to rapidly and efficiently generate mutant alleles of 15 genes in cultured somatic cells or human pluripotent stem cells, the latter for which we differentiated both the targeted lines and isogenic control lines into various metabolic

cell types. We demonstrate cell-autonomous phenotypes directly linked to disease_dyslipidemia, insulin resistance, hypoglycemia, lipodystrophy, motor-neuron death, and hepatitis C infection. We found little evidence of TALEN off-target effects, but each clonal line nevertheless harbors a significant number of unique mutations. Given the speed and ease with which we were able to derive and characterize these cell lines, we anticipate TALEN-mediated genome editing of human cells becoming a mainstay for the investigation of human biology and disease.

F-2031

OPTIMIZING NIS REPORTER GENE TECHNOLOGY FOR STEM CELL TRACKING APPLICATIONS

Hongtao Li[1], Patrycja Lech[1], **Holly Russell**[3], Lukkana Suksanpaisan[1], Brian Lenzmeier[4], Linh Pham[1], Michael Barry[2], Kah Whye Peng[1], Stephen J. Russell[1]
[A]Department of Molecular Medicine, [B]Department of Infectious Disease, [1]Mayo Clinic, Rochester, MN, United States, [2]Imanis Life Sciences, Rochester, MN, United States, [3]Department of Biology, Buena Vista University, Storm Lake, IA, United States

NIS, the thyroidal sodium iodide symporter, is the reporter gene of choice for studies seeking to determine the location, trafficking and long term fate of stem cells at serial timepoints in living animals or human subjects. In contrast to luciferase, NIS is a nonimmunogenic, nontoxic self-protein that is compatible with tomographic SPECT/CT and PET/CT imaging protocols employing clinically approved and readily available radiotracers. To address the concern that the NIS protein from a given species could be targeted and prematurely destroyed by the immune response of another species, we have made a panel of lentiviral vectors encoding pig, dog, mouse, rat and human NIS proteins. Each of these species-specific NIS vectors was shown to transfer a functional NIS protein into cultured cells, permitting them to efficiently concentrate radioactive iodide. To facilitate the customization and optimization of transduction protocols for each stem cell type of interest, from any animal species, we next generated a panel of bicistronic lentiviral vectors encoding both NIS and a selectable marker or fluorescent protein. Each of these vectors was shown to express a functional NIS protein and the expected selectable marker or fluorescent protein. We have also generated lentiviral vectors encoding an iodide sensitive yellow fluorescent protein (YFP H148Q/I152L) alone or with NIS as a non-radioactive option for measuring NIS bioactivity in cultured cells. YFP-H148Q/I152L fluorescence is quenched by iodide ions concentrated into NIS expressing cells, providing the basis for a fluorescence quenching assay to accurately and quantitatively indicate the level of cellular NIS expression. Using some of these tools, we optimized a transduction protocol to transfer the human NIS gene into human fat-derived mesenchymal stem cells (MSCs) and confirmed that these cells could be accurately and sensitively localized by radioiodine SPECT/CT imaging after they had been inoculated into living rodents. The tools and protocols that have been developed in this project are now being applied to stem cell tracking projects in larger animals, initially pigs.

F-2032

MANIPULATING THE CELLULAR MICROENVIRONMENT TO ENHANCE THE NEURONAL DIFFERENTIATION OF NEURAL STEM CELLS

Shreyas Shah, Aniruddh Solanki, Perry T. Yin, Ki-Bum Lee
Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ, United States

In recent years, RNA interference (RNAi) is emerging as an important tool for controlling gene expression in stem cell biology. RNAi involves using small interfering RNA (siRNA) to selectively silence genes or signaling pathways to control cellular behavior. However, the conventional methods used to deliver siRNA into stem cells result in significant cytotoxicity and undesirable side-effects. To this end, we have developed a nanotopography-mediated reverse uptake platform (NanoRU) to demonstrate a simple, non-toxic and highly effective technique for delivering siRNA into neural stem cells (NSCs). Our approach relies on improving the efficiency of siRNA delivery by simply altering the cellular microenvironment, wherein we introduce nanotopography to the underlying cell substrate to enhance cellular endocytosis. NanoRU consists of a self-assembled silica nanoparticle film coated with extracellular mat-

rix proteins and the desired siRNA of interest. We show that siRNA delivery to NSCs is dependent on the size of the nanoparticles forming the nanotopographical features and that only the siRNA molecules, and not the nanoparticles, are taken up by the NSCs. Furthermore, we use our platform to efficiently deliver siRNA against the transcription factor SOX9, which is known to act as a switch between the neuronal and glial fate of NSCs. The successful delivery of the siRNA and knockdown of SOX9 enhanced the neuronal differentiation and decreased the glial differentiation of the NSCs. This technology relies on the ability of the cells to sense the nanotopographical features and take up only the siRNA from its microenvironment, and it does not require the use of exogenous agents that can perturb the cells. NanoRU is a remarkable intracellular delivery platform which can truly complement conventional genetic manipulation tools in stem cell biology. Moreover, our results show great promise for designing nanostructured scaffolds to advance stem cell therapies.

F-2033

ONSET OF CIRCADIAN OSCILLATION OF PERIOD 2 COINCIDES WITH CELL DIFFERENTIATION IN MOUSE EMBRYONIC STEM CELLS

Jacob Nobel[1], Maureen Kearnan[1], MNT Nor-Ashikan[2], Steve Sheridan[3], Fred Davis[4], **Ann Kiessling**[1]
[1]Bedford Research Foundation, Bedford, MA, United States, [2]Physiology, University Technology MARA, Selangor, Malaysia, [3]Center for Human Genetic Research, Massachusetts General Hospital/HMS, Boston, MA, United States, [4]Biology, Northeastern University, Boston, MA, United States

The influence of light/dark cycles on physiological processes is mediated through the suprachiasmatic nucleus (SCN), a small area in the hypothalamus that receives direct retinal innervation. The SCN entrains 24-hour, circadian, rhythms to the light dark cycle due to the interaction of a set of transcription factors, the core of which are Clock and Arntl (Bmal) whose dimerization promotes the expression of their inhibitors, Period and Cryptochrome. The Period/Cryptochrome dimer inhibition of Clock/Arntl, brings about a drop in Period/Chryptochrome, releasing Clock/Arntl from inhibition, so the cycle begins again. Evidence to date indicates that approximately 10% of gene expression is under circadian control. Peripheral tissues maintain their individual circadian oscillations in gene expression, unique to the tissues, but over all synchronicity of body functions is orchestrated by the SCN.
The discovery that 8-cell human embryos, but not cultured human embryonic stem cells, expressed the core circadian oscillators suggested that circadian signals may play a role in early embryo cleavage. One possible role is stabilizing cell division since the key gap 1 cell cycle checkpoint, Rb, is silent in early embryos.
To explore the possible role of the core circadian oscillators in early development, we derived two embryonic stem cell lines from the mouse transgenic for Period 2 linked to the reporter gene Luciferase, Per2Luc. We characterized the two lines with respect to growth characteristics, pluripotency, potential to develop all 3 germ layers in embryoid bodies, ability to undergo directed differentiation into neuronal and cardiomyocyte cell types, and light production in the presence of luciferin substrate.
We were unable to detect light produced by individual embryos, or cell colonies with fewer than 50,000 cells. Pluripotent cell cultures expanded on gelatin with LIF and mouse fibroblast conditioned medium (MFCM) produced 30 counts of light per 100,000 cells, detected in a sensitive luminometer in multiple experiments, thus indicating the constitutive expression of the Period2-Luc construct. Withdrawal of LIF and MFCM, and brief induction by retinoic acid, resulted in cultures with mixed cell types, including neuronal and beating colonies of cardiomyoblasts. Within five weeks of differentiation, luminometer tracings of light production showed a 24-hour rhythmicity with peaks approximately two-fold higher in light production than valleys. Once entrained, the cells maintained their circadian oscillations following trypsinization and re-plating into new dishes.
These new cell lines are valuable reagents for studying many aspects of the role of the core circadian oscillators during development. The results raise several questions: (1) is the low level of light in pluripotent cell cultures due to low level expression of Per2 in all cells or expression of Per2 in only a few cells? (2) Does the onset of rhythmicity indicate up regulation of Per2 specifically in differentiated cells? (3) What are the intercellular signals that bring a diverse population of differentiating cells into synchrony? (4) is Per2 oscillation a characteristic of one cell type in

the culture, but not all cell types? Answers to these questions require the development of highly sensitive, optically accurate microscopy.

F-2034

CHARACTERIZATION OF THREE EARLY PASSAGE CLINICAL GRADE HUMAN EMBRYONIC STEM CELL LINES

Seth M. Taapken[1], Lynn M. Boehnlein[1], Nicole J. George[1], Lori A. Norkosky[1], Tenneille E. Ludwig[2], Benjamin S. Nisler[1], Karen Dyer Montgomery[2], Jeffrey M. Jones[3]
[1]WiCell Research Institute, Madison, WI, United States, [A]Stem Cell and Regenerative Medicine Center, [B]Obstetrics and Gynecology, [2]University of Wisconsin, Madison, WI, United States

Efficient and successful translation of pluripotent stem cell research into human therapeutics will require high quality, well characterized cell lines capable of supporting development from research through human clinical trials. Additionally, the ability to use cells not exposed to animal products during the initial derivation and expansion offers a significant advantage by simplifying regulatory requirements. We have recently derived three new human embryonic stem cell (hESC) lines, WA25, WA26 and WA27, using chemically defined, feeder-independent and xeno-free conditions (modified Essential E8 medium on the VTN-N variant of vitronectin). Embryos used in the derivation of these lines were recovered prior to 2005, making these three cell lines exempt from FDA 21 CFR Part 1271 requirements for donor eligibility determination, further easing regulatory compliance.
Initial characterization of WA25, WA26 and WA27 included karyotype (normal), identity by STR, HLA and ABO blood typing, sterility (sterile), mycoplasma assessment (negative), and hESC marker expression (Oct3/4, SSEA3, SSEA4, Tra 1-60, Tra 1-81 and SSEA1) by flow cytometry (pluripotent). Testing for human infectious agents (Charles River, Human Comprehensive Virus Panel) was also performed (negative for human viruses). Population doubling time for WA25, WA26 and WA27 was determined to be 24.6±2.1, 20.9±2.1 and 18.2±2.1 hours, respectively. When each cell line was individually injected into SCID-Beige mice, teratomas consisting of all three germ layers were obtained. Additionally, each of these cell lines were maintained in culture for more than 40 passages without significant change (data not shown).
Further characterization utilizing higher resolution methods will be required to ensure the safety of these cells prior to transplantation in humans. Using the Illumina HumanCytoSNP-12 v2.1 and Illumina's Genome Studio software we demonstrated that these clinical-grade hES cell lines (WA25 p7, WA26 p8, WA27 p8) carried few copy number changes and none greater than 250kb when compared to a diverse set of over one-hundred samples from the Caucasian (CEU), Asian (CHB+JBT), and Yoruban (YRI) HapMap populations. Loss of heterozygosity (LOH) was also cataloged for each cell line.
These results demonstrate genomic stability and differentiation potential of the WA25, WA26 and WA27 cell lines produced by WiCell, and validate their potential usefulness for human clinical applications.

F-2035

GENOME EDITING OF HUMAN PLURIPOTENT CELLS USING TALEN AND CRISPR/CAS SYSTEMS

Qiurong Ding, Chad Cowan, Kiran Musunuru
Harvard department of stem cell and regenerative biology, Cambridge, MA, United States

The use of genome editing to generate isogenic cell lines with and without disease mutations allows for rigorous human disease modeling, either by introducing mutation(s) into wild-type human embryonic stem cell (hESC) lines or induced pluripotent stem cells (hiPSCs) or by "curing" the mutation(s) in patient-specific hiPSCs. Among various technologies, transcriptional activator-like effector nuclease (TALEN) and clustered regularly interspaced short palindromic repeats/Cas9 nuclease (CRISPR/Cas) systems have emerged as rapid and efficient means by which to perform genome editing in human pluripotent stem cell lines. Both TALENs and CRISPRs are easy to engineer to induce double-strand breaks at specific genomic sequences, but they induce double-strand breaks at the sites by very different mechanisms. To assess which system is more efficient in human pluripotent stem cells, we targeted the same

genomic sites in more than a dozen genes using both TALENs and CRISPRs in the same hESC lines or hiPSC lines. We compared both the efficiencies of generating small insertions or deletions (indels) by the error-prone process of non-homologous end-joining (NHEJ), which can be used for gene knockout, and the efficiencies of knocking in point mutations by homology-directed repair (HDR) using single-stranded DNA oligonucleotides (ssODNs) as templates. With sequencing of genome-edited clones, we also compared the rates of off-target effects produced by the TALEN and CRISPR systems. Besides establishing that we can rapidly and efficiently target human pluripotent stem cells using both systems, we provide comparative information that should prove useful to investigators in choosing which genome-editing system to use for various disease-modeling and clinical applications with human pluripotent stem cells.

F-2036

SCHRÖDINGER REVISITED: UNDERSTANDING DEVELOPMENTAL BIOLOGY AND DISEASE THROUGH QUANTUM MECHANICAL MODELLING TECHNIQUES

Alan L. Jakimo
Sidley Austin LLP, New York, NY, United States

String theorists point to mutations in DNA as a metaphor for quantum fluctuations in cosmological space-time settings. This should remind us of Schrödinger's 1944 book What is Life? - a work that propelled Watson and Crick in 1953 to successfully model DNA's structure. Over ensuing decades the genetically-programmed developmental biology of a range of organisms - from the most simple to the most complex (including humans) - has been dissected and presented with "circuit" diagrams showing molecules and molecular complexes connected by lines with activation arrowheads and inhibition bars perpendicular to the connectors. This linear circuit approach with various positive and negative feedback loops, however, may not capture the stochastic complexity that attends developmental biology and disease progression. Toward the objective of better models, quantum physicists and life scientists are actively collaborating. Here proposed is a topological approach to this problem of modeling developmental biology and related disease processes that borrows from string theorists a set of mathematical constructs called Calabi-Yau Manifolds.
The approach proposed here involves the following steps: 1) constructing a cellular-level map of the developmental pathways followed by individual cells in the blastocyst; 2) associating as a dynamic object the polycomb with each cell in the blastocyst cell pathway map; 3) associating with the polycomb for each such cell an epigenome topology; 4) describing the intracellular and intercellular space-time dynamics of the polycomb and the associated epigenome topology in terms of stochastic processes taking place at the quantum mechanical level; and 5) testing the fit of various Calabi-Yau Manifolds (CYMs)- the work horse of string theorists - to the structure and behavior of the multi-dimensional object resulting from the first four steps.
Results of this approach at this point are heuristic at best and require refinement, but nonetheless suggest that a rigorous program should be pursued collaboratively to model developmental biology with CYMs.

F-2037

DISCOVERING SIGNALING PATHWAYS AND DRUGGABLE TARGETS IN DIABETIC DISEASE PATHOPHYSIOLOGY USING IPSCS DERIVED CELLULAR MODELS

Roberto Iacone[1], Christoph Patsch[1], Jacques Bailly[1], Eva Christina Thoma[1], Guillemette Duchateau-Nguyen[1], Tobias Bergauer[1], Klaus Christensen[1], Lin Sun[2], Dorothee Kling[1], Gregor Dernick[1], Paul Huang[2], Ken Chien[3]
[1]F. Hoffmann - La Roche, Basel, Switzerland, [2]Cardiovascular Research Center and Cardiology Division, Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States, [3]Harvard Stem Cell Institute, Boston, MA, United States

The Number of people with diabetes mellitus worldwide has more than doubled over the past three decades and is projected to constantly rise; this has brought a significant increase in the prevalence of cardiovascular and meta-

bolic disorders. Despite huge investments by the pharma industry, the development of new medicines for such disorders has proven to be challenging due to the use of cellular models which recapitulate some subsets of the specific features of the human disease.
Herewith, we describe how the advent of the patient-specific iPSC technology in combination with the *in vitro* mimicking of the "diabetic microenvironment stressors" allows the study of signaling pathways and response to selective drugs in desired cellular models.
To resemble cellular features of cardiomyocytes (CMs) from hearts affected by diabetic cardiomyopathies, iPSCs derived-CMs are forced to use a complete oxidative metabolism and then switched to a glycolytic ones in combination with other stressors. The mentioned metabolic switch, beyond a role in energetic support, represents a valuable *in vitro* model to investigate the crosstalk between metabolic flux, cellular signaling and epigenetic regulation of cell fate. Progressive switch to a hyper-glycolytic metabolism determines a disarray of sarcomeric structures, defects in intercellular calcium kinetics and up-regulation of clinical relevant cardiovascular biomarkers. To investigate the mechanisms of endothelial dysfunction, documented in Type 2 Diabetes (T2D) macro-vascular complications, we generated patients specific iPSCs lines for 4 diabetic extreme patients with accelerated vs. slower progression to Coronary Artery Disease (CAD). The patient iPSCs lines have been differentiated in endothelial cells (ECs) for disease modeling and drug discovery. Using expression profiling (RNA sequencing, secretome analysis) of patient-ECs and exposure to inflammatory cytokines, we can demonstrate that atherosclerosis relevant signaling pathways are active in this cellular model. The comparison between the two extreme phenotypes may ultimately elucidate novel mechanisms implied in atherosclerosis and new targets to be pursued in a drug discovery campaign. Thus, the use of functional iPSCs derived cellular models in combination with disease modeling assays may represent a powerful tool to recapitulate specific features of human cardiovascular and metabolic disorders.

F-2038

THE STEM CELL COMMONS: A RESOURCE FOR DEVELOPING AND SHARING DISEASE MODELS

Shannan J. Ho Sui[1], Sudeshna Das[2], Emily Merrill[2], Ilya Sytchev[1], Nils Gehlenborg[3], Richard Park[3], Stéphane Corlosquet[2], Psalm Haseley[3], Terah A. Hansen[1], John Hutchinson[1], Oliver Hofmann[1], Peter J. Park[3], Winston Hide[1]
[1]Biostatistics, Harvard School of Public Health, Boston, MA, United States, [2]MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, Cambridge, MA, United States, [3]Center for Biomedical Informatics, Harvard Medical School, Boston, MA, United States

Assays of biological activity are increasingly driven by the proliferation of technologies, platforms and methods in omics biology. In turn, data analysis and interpretation have become more multidisciplinary and complex. Sharing of expertise, data, and models of disease needs to move beyond the bottlenecks of email and spreadsheets. To leverage the potential of omics integration, we have established a platform that provides the ability to find similar classes of experiments and that standardizes analysis approaches so that researchers can directly compare and integrate their results with other experiments and disease models.
The Stem Cell Commons is a web-based resource for processing, analysis and sharing of stem cell data (stemcellcommons.org). It has been designed to aid knowledge discovery and community development in stem cell science, and ultimately, provide a platform for in silico analysis of disease models. Based at the Harvard Stem Cell Institute (HSCI), the resource hosts data from HSCI researchers and public data of significant interest to the community. Over 160 genomics studies focused on stem cell and cancer samples (primarily leukemia and glioma) are available for experiments performed using microarray and next generation sequencing technologies in human, mouse, rat and zebrafish tissues. All data is carefully curated and annotated with controlled vocabularies and standard terminologies. Microarray data in the system have been consistently processed using a novel tool called PathPrint that enables comparisons of pathway signatures across experiments. PathPrint allows users to identify experiments with similar pathway activities and enables classification of samples based on function.
We believe that the Stem Cell Commons, with its open source environment for sharing stem cell datasets, analysis tools and code, will spur new discoveries- and new points of interest- among stem cell researchers.

F-2041

ENCODING OF TEMPORAL SIGNALS BY THE TGF-β PATHWAY AND IMPLICATIONS FOR CELL FATE DECISIONS

Benoit` SORRE, Aryeh Warmflash, Ali H. Brivanlou, Eric D. Siggia
The Rockeeller University, New York, NY, United States

The TGF-β signaling pathway is used iteratively throughout development to guide cell-fate decisions. We examined the TGF-β response in individual living cells with the goal of understanding pathway dynamics and their role in cell-fate specification. We used the myoblastic C2C12 cell line as a paradigm for studying signaling dynamics and fate decisions. We found that R-Smads stably translocate to the nucleus and report the ligand level. In contrast, Smad4 nuclear localization is confined to short pulses that coincide with transcriptional activity. We then took advantage of a microfluidic cell culture system to deliver complex temporal stimuli and further characterize signaling dynamics. By increasing the ligand with variable speeds to the same final concentration, we showed that the pathway responds to the rate of ligand delivery. If the speed of delivery is sufficiently slow, the response is inhibited. Similarly, we showed that as a consequence of transient nature of the pathway dynamics, output is increased if the ligand is delivered in repeated short pulses. Repeated pulses were also more effective than constant stimulation at inhibiting myotube differentiation. Finally, we evaluated TGF-β dynamics in the Xenopus embryo. Smad4 shows stereotyped, uncorrelated bursts of nuclear localization while activated R-Smads are uniform. Altogether, these results show the importance of considering pathway dynamics in order to understand how morphogens guide cell-fate decisions.

F-2042

A NOVEL PEPTIDE FOR STEM CELL CULTURE

Sarah A. Ritson, Lisa Mohamet, Natalie Miazga, Zahra Y. Abdalla, Chris M. Ward
Stem Cell Research Group, University of Manchester, Manchester, United Kingdom

Stem cells have the capacity to form any cell within the adult body which makes them excellent candidates for tissue engineering applications. However, before stem cell technology can be fully realised, significant hurdles remain to be overcome, including the development of cost-effective, scalable cell culture methods and the ability to fractionate populations of cells suitable for therapy. Many current techniques used for this purpose involve labelling cells and sorting them individually using fluorescence activated cell sorting (FACS). A system which enables the fractionation of large quantities of stem cells (or their differentiated progeny) according to their receptor interactions with extracellular matrix (ECM), or their reaction to topographies/ mechanical stimuli that mimic ECM, would open up new possibilities for the high-throughput purification of distinct cell populations.
An essential component of cell sorting is the disaggregation of cell populations to provide a single cell suspension. However, loss of cell-cell contact in stem cells can lead to decreased growth potential and induce apoptosis. The major cell adhesion molecule associated with epithelial cell-cell contact is E-cadherin. We have previously shown that abrogation of E-cadherin in human ES cells using an inhibitory antibody leads to decreased proliferation in monolayer culture and increased cell death in suspension. We have utilised a novel peptide inhibitor of E-cadherin (ePEP) and assessed proliferation and cell death in a range of cell lines. We show that ePEP leads to loss of cell-cell contact in human and mouse ES cells and human breast and oral epithelial cell lines. Furthermore, ePEP treatment leads to increased survival in these cell lines in both monolayer and suspension. Microarray analysis demonstrates limited gene transcript changes in human cells treated with ePEP and confirms that the process is fully reversible. ePEP therefore provides a useful tool for the culture of human epithelial cell lines and in providing single cell suspensions for subsequent cell fractionation.

F-2043

GLOBAL IDENTIFICATION OF ACTIVE CIS-REGULATORY MODULES IN MOUSE EMBRYONIC STEM CELLS

Matthew J. Murtha, Zeynep Tokcaer-Keskin, Zuojian Tang, Yatong Wang, Stuart Brown, Claudio Basilico, Lisa Dailey
NYU Medical Center, New York, NY, United States

Mammalian embryonic stem cells (ESCs) represent a unique cell state characterized by the properties of self-renewal and pluripotency. These characteristics of cell identity are largely governed by different sets of core transcription factors (TFs) that serve to establish cell-specific chromatin landscapes and hierarchies of gene expression through DNA binding. Thus a full understanding of cell identity requires the identification of not only key TFs but also of the key, *ie*. functional, target DNA elements comprising their distinct transcriptional circuitries. The majority of analyses in ESC have focused on ChIP-based binding studies of Oct4, Sox2, and Nanog however it is difficult to ascertain which DNA targets are governing active transcription. Further, it is likely additional factors contribute to pluripotency that remain largely uncharacterized, and little is known regarding the TFs and target DNAs mediating the earliest events in the lineage specification of ES cells.
To address this, we have developed a novel functional screen allowing high-throughput identification of active stage-specific promoter and enhancer elements from ESCs and ESCs undergoing differentiation. The screen uses a simple method for isolating short (~150bp) nucleosome free regions (NFRs) from open ESC chromatin to generate lentiviral libraries in which individual ESC-derived NFRs drive expression of a GFP reporter gene. This allows the functional interrogation of these DNAs for promoter/enhancer activity upon transduction and differential activation of GFP in ES- and differentiated cells. With this method we have identified a multitude of elements throughout the murine genome that are active in mESCs. Transcriptional activation measured by an independent assay, as well as histone modifications and expression levels of genes proximal to these elements are consistent with the notion that these elements represent active cis-regulatory modules, potentially with stage-specific activity. Distal and stage specific elements can be subjected to motif analysis to identify potential cognate TFs. Global identification of active elements will permit the addition of functional information to the annotation of the mouse genome, which can then be integrated with other high-throughput mapping data, facilitating the development of robust models of ES transcriptional networks and the identification of novel stage-specific TFs.

F-2044

EFFICIENT AND SCALABLE ENDOTHELIAL CELL DIFFERENTIATION METHOD FROM HUMAN INDUCED PLURIPOTENT STEM CELLS BASED ON 2-D MONOLAYER AND SERUM-FREE CULTURE

Takeshi Ikuno[1], Hidetoshi Masumoto[1], Akira Marui[2], Tadashi Ikeda[2], Ryuzo Sakata[2], Jun K. Yamashita[1]
[1]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [2]Department of Cardiovascular Surgery, Kyoto University Graduate School of Medicine, Kyoto, Japan

BACKGROUNDS: Efficient and scalable endothelial cell
(EC) differentiation system from human induced pluripotent stem cells (hiPSCs) is
important for vascular regenerative medicine and disease modeling. We
previously established a mouse pluripotent stem cell differentiation system
that reproduces early vascular development using vascular endothelial growth
factor (VEGF) receptor-2 (VEGFR2)-positive mesoderm cells as common vascular progenitors
(*Nature* 2000; *Circulation* 2008). We found that VEGF is essential to induce ECs, and 8-bromo
cyclic adenosine monophosphate (cAMP) enhances VEGF-induced EC differentiation
(*Arterioscler Thromb Vasc Biol* 2006; *Blood* 2009). We also established an efficient monolayer high-density
culture-based cardiomyocyte (CM) differentiation protocol from hiPSCs (with
modifications on a directed differentiation method for human embryonic stem
cells (Laflamme, Nat Biotechnol, 2007))(*PLoS*
One 2011). Here we attempted to potently induce ECs from hiPSCs based
on this differentiation protocol by directing mesoderm-stage cells to ECs with
VEGF and cAMP activation. METHODS & RESULTS: Various concentrations
of VEGF and
8-bromo cAMP were supplemented at time points around the possible mesoderm
emergence, then efficiency of EC induction was evaluated with flow cytometry. Vascular
endothelial (VE)-cadherin-positive EC population was significantly increased with
addition of VEGF (100ng/ml, from differentiation day 4) and cAMP (1mM, from day

4 to 6) compared to those with VEGF supplementation alone, or those with no VEGF and cAMP (56.2±12.5% vs 11.8±7.2% vs 2.3±2.4% of total cells, P=0.000017, n=4, analyzed on differentiation day 9). Calculated EC count was also notably increased with combined VEGF and cAMP treatment ($1.76±0.70×10^5$ vs $4.9±3.3×10^4$ vs $9.8±10.4×10^3$ cells / cm^2 culture surface, P=0.0022, n=4, day 9). On the other hand, CM population inversely decreased according to the treatment (20.0±13.2% vs 47.6±25.7% vs 55.9±12.1% of total cells, P=0.049, n=4, day 9).
CONCLUTIONS: Here we reported an efficient and scalable EC differentiation method from hiPSCs based on 2-D monolayer and serum-free culture. We controlled direction of differentiation with stage-specific supplementation of VEGF and cAMP. We have developed a valuable technological basis for vascular regenerative therapy and vascular disease modeling using disease-specific hiPSC-derived ECs for the innovation of drug discovery.

F-2045

ENZYME FREE PASSAGE OF HUMAN PLURIPOTENT STEM CELLS

Kiyoshi Ohnuma[1], Ayaka Fujiki[2], Satoshi Koike[1], Yohei Hayashi[3], Yuzuru Ito[4], Yasuko Onuma[4], Techuan Chan[3], Tatsuo Michiue[3], Miho K. Furue[2], Makoto Asashima[4]
[1]Bioengineering, Nagaoka University of Technology, Niigata, Japan, [2]Laboratory of Stem Cultures, National Institute of Biomedical Innovation, Osaka, Japan, [3]Department of Life Sciences, The University of Tokyo, Tokyo, Japan, [4]Research Center for Stem Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST), Ibaraki, Japan

Human pluripotent stem cells (hPSCs) have increased the possible applications of stem cell research in biology and medicine. Enzymatic digestion during the passage of hPSCs causes cell damage and instability due to batch variations. Here, we report that serum- and feeder-free conditions enable cell-ECM (extracellular matrix) and cell-cell bindings to be independently controlled without enzymes. The hPSCs were harvested as large colonies unlike those using buffer solutions with ethylene-diamine-tetraacetic acid (EDTA). The passage conditions could be applied to laminin-, vitronectin-, and fibronectin-coated culture dishes. The addition of trypsin caused less cell adhesion. The cells adhered more by reducing the amount of trypsin. The hPSCs could be maintained for long periods (more than two months) without karyotypic changes. Our results suggest that serum- and feeder-free conditions allow hPSCs to be cultured in the absence of enzymes with minimal cellular damage.

F-2046

GENERATION OF THE HUMAN ES CELL LINE DRIVEN BY DIOXIN RESPONSIVE REPORTER GENE

Seiichiroh Ohsako
The University of Tokyo, Faculty of Medicine, CDBIM, Tokyo, Japan

Previously in the course of research to establish an effective alternative developmental toxicity test using human embryonic stem cell (hESC), we reported the toxicological responses of hESC and subsequent differentiated cells against the most toxic environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). During differentiation process of hESC to neuronal cells in vitro, CYP1A1 gene that is a biomarker to TCDD were induced only in embryoid body formation stage, but not in ES cell and matured neuronal cell stages. The higher sensitivity of cells in embryoid body seems to cause the inhibition of differentiation of endoderm cells and lead the higher rate of ectoderm cell differentiation. These findings suggested that rather than pluripotent and differentiated central nervous cells, specific stage cell type of early differentiation are more susceptible to TCDD. Here we have generated a hESC

lines carrying CYP1A1 gene driven EGFP reporter to enable to monitor real time induction level of dioxin biomarker during various phases of cellular differentiations.
Mouse CYP1A1 promoter region (-1500bp) containing eight xenobiotic responsive elements (XREs) connected to EGFP with neomycin resistant gene cassette was transfected to KhES1 cells with Lipofectamine LTX and then selected by 10 mg/mL G418 on the SNL feeder cells. The generated KhES1-CYP-EGFP cells were exposed to TCDD (0.1, 1, 10 nM) for 24 hrs. At low and middle doses (0.1 and 1 nM), EGFP expression were similar to that of control DMSO group. However at high dose (10 nM) EGFP expression was clearly enhanced, indicating that the reporter transgene is able to be activated in pluripotent stage by TCDD. The discrepancy of EGFP induction and endogenous human CYP1A1 gene silence may be due to difference of epigenomic status between transgene and native endogenous genes.
The research was supported by Grant-in-aid from the Ministry of Health, Labour and Welfare of Japan.

F-2047

COMPARATIVE ANALYSIS OF EX VIVO EXPANDED HUMAN CD34+ HEMATOPOIETIC STEM CELLS ON NANEX™ NANOFIBER PLATES

Mariluz M. Henshaw[1], Stephen E. Fischer[2], Yukang Zhao[2], Linda L. Kelley[3], Michael Boyer[1]
[1]Cell Therapy and Regenerative Medicine Program, University of Utah, Salt Lake City, UT, United States, [2]Arteriocyte, Cleveland, OH, United States, [3]Cryo-Cell International, Inc., Oldsmar, FL, United States

Hematopoietic stem cells (HSC) are increasingly used in treating a wide variety of malignant and degenerative diseases with promising results. Cells expressing CD34 (CD34+) in bone marrow (BM), mobilized peripheral blood (PB) and umbilical cord blood (UCB) identify a rare cell population with HSC progenitor characteristics. The number of CD34+ HSC available is invariably limited and for some clinical applications, *ex vivo* expansion is required to generate the cell numbers needed for successful transplant. The development of *ex vivo* culture systems that enable efficient expansion and maintenance of CD34+ HSC is a crucial step to harnessing its full potential for use in cell-based therapies.
Arteriocyte recently launched an *ex vivo* culture system, the NANEX™ HSC Expansion Kit. The NANEX™ coating is a chemically-modified polymeric nanofiber mesh that forms a 3D scaffold upon which the cells adhere. This 3D scaffold partially mimics the BM microenvironment promoting cultures of CD34+ cells on NANEX™ plates to efficiently expand and maintain the CD34+ phenotype. Most of these studies were performed using CD34+-selected cells from UCB.
In this study, we independently evaluated Arteriocyte's NANEX™ HSC Expansion Kit as an *ex vivo* culture system using cryopreserved CD34+-selected cells from UCB and mobilized PB cultured in parallel. Three samples each of frozen CD34+-selected cells from UCB and mobilized PB were thawed and cultured in parallel for eight days on NANEX™-coated or regular 6-well tissue culture (TC) plates. The cells were maintained in serum-free HSC expansion medium and optimized cytokine cocktail provided in the kit. Cell counts, flow cytometry analyses and colony-forming cell (CFC) assays were done both at initiation and at termination of cell cultures.
There was on average, a 124-fold expansion of UCB CD34+ cells and four-fold expansion of mobilized PB CD34+ cells on NANEX™-coated plates after the eight-day culture period. In contrast, UCB CD34+ cells cultured in uncoated TC plates had a 25-fold expansion and mobilized PB CD34+ cells did not proliferate at all. Flow cytometry analyses of cells harvested from NANEX™ plates showed that UCB CD34+ cells maintained a larger percentage of CD34+ cells (average = 24.5 %) compared to mobilized PB CD34+ cells (average: 9.7 %). UCB and mobilized PB CD34+ cells harvested from uncoated TC plates had markedly lower percentages of CD34+ cells (8.5 % and 5.0 %, respectively). There was no significant difference in CFC content (CFC per 500 CD34+ cells plated) between CD34+ cells used to initiate the cultures and the cells harvested after the eight-day culture period, indicating that NANEX™ culture maintains colony-forming cells.
This study validates the use of NANEX™ HSC Expansion Kit as an efficient system for the *ex vivo* expansion of UCB CD34+ cells. Expansion of mobilized PB CD34+ cells was likewise enhanced but not to the same degree. Mobilized PB CD34+ cells are known to be less proliferative than UCB CD34+ cells, and efforts to expand these cells *ex vivo*

have not been as successful to date. It is likely that mobilized PB CD34+ cells might require other growth factors and/or interactions with the microenvironment for their optimal growth. Nonetheless, the results of these initial *ex vivo* experiments look promising and further studies are warranted.

F-2048

MINING THE PROTEOME OF NORMAL BREAST STEM CELLS SUB-POPULATIONS FOR DISCOVERY OF STEM CELL SPECIFIC PROTEIN BIOMARKERS

Ayodele Alaiya[1], Hazem Ghebeh[2], Ghida Majed Sleiman[2], Zakia Shinwari[1], Eman Barhoush[1], Manogaran Pulicat[2], Amer Al-Mazrou[2], Asma Tulbah[3], Taher Al-Tweigeri[4], Khaled Al-Faqeeh[5], Chaker Adra[2]

[A]Proteomics Unit, Stem Cell & Tissue Re-Engineering Program, [B]Stem Cell & Tissue Re-Engineering Program, [C]Department of Pathology & Laboratory Medicine, [D]King Faisal Cancer Center, [E]Department of Surgery, [1]King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia

Background/Aims

There is evidence for the existence of adult stem cells in the normal human breast. Currently panel of surface markers of differentiation including CD44, CD24, detoxifying enzymes like aldehyde dehydrogenate (ALDH), and epithelial cell adhesion molecule (EpCAM) are routinely used, individually or in combination to characterize breast normal stem cells. The existence of overlap in specificity of the different markers in typing stem cells in the adult normal breast tissues has been documented. This study aimed to identify and characterize novel proteins as biomarkers for normal stem/progenitor cells using expression proteomics approach.

Materials/Methods

Normal breast tissue was digested and extraction of the mammary epithelial cells was done. Lymphocytes, fibroblast, and endothelial cells were depleted. The isolated epithelial breast cells were sorted by fluorescence assisted cell sorter (FACS) combined with standard breast stem cell markers ALDH+, $CD44^{high}/CD24^{low}$. Further enrichment was done by sorting for CD90+ fraction of $Ep\text{-}CAM^{low}$/CD49f+ cells, and was compared with $CD90^{neg}$ cells. Whole cell lysates of sorted cells were subjected to proteome analysis by 2-DE, MALDI-TOF-MS and LC/MS/MS.

Results

We observed high degree of homogeneity in global protein expression profiles from CD90+ and $CD90^{neg}$ cells of the $Ep\text{-}CAM^{low}$/CD49f+ cells. Due to low protein yield from few numbers of sorted cells, we subjected whole cell lysates of only the Ep-CAMlow/CD49f+ cells to LC/MS/MS analysis towards identification of new surface markers**.** Approximately 190 protein isoforms representing only 90 unique protein species were identified by LC/MS/MS. Only 4 of the 90 proteins are cell surface proteins. Among the identified protein are ABCC6 ATP-binding cassette, sub-family C (CFTR/MRP), member 6 , FMS-Related tyrosine kinase 4 , Annexin A2, Killer cell lectin-like receptor subfamily C, member 1, Moesin and Neurexin 3.

Conclusions

We have identified new proteins that might be specific markers for identification of normal human mammary stem cells. One of them is ABCC6 a member of the ATP binding cassette protein like G2 (ABCG2), which currently is being used alone or in combination with EpCAM to type CSCs All identified 6 proteins are cell surface that can be used for sorting of alive cells and further functional analysis of normal adult breast stem cells. The results highlight the power of proteomics for discovery of biomarkers for breast cancer stem cells.

Further discussion on technical challenges on the path to defining breast cancer stem cells and data supporting validation of the identified proteins in clinical samples will be presented.

F-2051

MUC1* LIGAND, NM23, IS A NOVEL GROWTH FACTOR THAT REVERTS UNMODIFIED HUMAN STEM CELLS TO THE NAïVE STATE IN A BFGF-FREE, FEEDER-FREE DEFINED SYSTEM

Cynthia C. Bamdad[1], Benoit J. Smagghe[1], Andrew K. Stewart[1], Mark G. Carter[1], Laura M. Shelton[1], Klyle J. Bernier[1], Eric J. Hartman[1], Amy K. Calhoun[1], Vasilios M. Hatziioannou[1], Gabriele Gabriele Lillacci[2], Brian A. Kirk[1], Kenneth S. Kosik[1]

[1]Minerva Biotechnologies, Waltham, MA, United States, [2]Department of Mechanical Engineering, University of California at Santa Barbara, Santa Barbara, CA, United States

We report that a single growth factor, NM23, is sufficient for reverting unmodified human stem cells to the naïve state. Pure populations of unmodified human stem cells were generated by culturing the cells in NM23 in an bFGF-free, feeder-free, defined system. Characterization of these cells showed that they were in the pre-X-inactivation state (XaXa), as evidenced by visualizing the pattern of tri-methylated (K27) Histone 3. These cells also expressed high levels of the genes associated with the naïve state and low levels of the genes associated with the primed state. Consistent with another characteristic of human stem cells in the naïve state, they tolerated serial dissociation using trypsin, proliferate even when plated at very low densities and had increased single cell cloning efficiency comparable to that of naïve murine stem cells. Culture in NM23 media differentiate normally and showed no karyotypic instability. Exposure to bFGF caused our naïve cells reverted to the primed state (XaXi) after only 4 passages.

When allowed to passively differentiate, NM23-cultured stem cells showed no germline preference. However, they differentiated in a coordinated way that was not observed for stem cells cultured in bFGF-containing media. For example, embryoid bodies that stained positive for Nestin had over 90% Nestin-positive cells. Conversely, bFGF-grown cells formed embryoid bodies, each comprised of equal numbers of cells differentiating down the 3 germlines.

Mechanism: Undifferentiated human stem cells express MUC1*, a cleaved form of the MUC1 transmembrane protein. However, as soon as stem cells initiate differentiation, MUC1 cleavage ceases and it reverts to the full-length quiescent state. Cleavage and release of the bulk of its extra cellular domain unmasks the binding site for MUC1*'s activating ligand NM23, which is secreted by pluripotent stem cells. NM23, regulates both pluripotency and differentiation, wherein whether it promotes pluripotency or differentiation depends on the multimerization state of the protein. NM23 *dimers* cooperatively bind to and dimerize the extra cellular domain of the MUC1* growth factor receptor. NM23-MUC1* complexes are internalized and translocated to the nucleus, where we speculate one or both function as transcription factors for promotion of stem-like growth. Competitive inhibition of the NM23-MUC1* interaction induced differentiation and a spike in the expression of miR-145, which signals a cell's exit from pluripotency. Conversely, NM23 *hexamers* do not bind to MUC1*. When added to undifferentiated stem cells, NM23 hexamers induce differentiation. We cultured cells in NM23 variants that were dimers and unable to form hexamers.

Model: NM23 exists as a monomer, dimer, tetramer or hexamer depending on concentration. Undifferentiated stem cells secrete NM23 and in dimeric form they bind to MUC1* and promote pluripotent stem cells growth. As they approach some critical cell density when stem cells in nature would initiate differentiation to develop into an embryo, the local concentration of NM23 in the surrounding media consists primarily of hexamers, which trigger differentiation. Discrete multimers, like the NM23 dimers and hexamers that exert opposite effects are able to exercise exquisite control over the pluripotency state and constitute an ON/OFF switch. Over a very narrow range of concentrations, NM23 switches from a differentiation repressor to a differentiation inducer, making NM23 the "pluripotency switch".

F-2052

INDIRECT REGULATION OF FLOW STIMULATED ENDOTHELIAL CELLS ON MURAL NEURAL PROGENITOR CELLS

Courtney M. Dumont, Maureen R. Newman, Nadeem H. Kazi, Evan Bowman, Guohao Dai, Deanna M. Thompson
Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY, United States

Introduction: Neural stem cells (NSCs) reside in many neural tissues but neurogenesis is most abundant in highly vascularized regions of the brain. Vasculature is a common element of many stem and progenitor cell microenvironments providing extracellular matrix (ECM) and soluble factors. Endothelial cells (ECs) comprising microvascu-

lature are known to promote NSC and neural progenitor cell (NPC) proliferation and inhibit differentiation directly via cell-cell contacts and indirectly through ECM protein and soluble factors. To date, NPC-EC *in vitro* models use statically-cultured EC, however, dynamically cultured EC are phenotypically different, exhibiting profound changes in soluble factor release profile (e.g. bFGF and EGF), which are known to influence NPC proliferation and ECM production. We developed an in vitro model to dynamically culture EC and collect soluble factors and insoluble ECM. Significant increases in bFGF and EGF were found in both isolated fractions. We evaluated cell response to these dynamically generated EC-factors in comparison to traditional static factors.

Methods: *EC Factors* –Confluent EC monolayers were exposed to static or dynamic culture (10 dynes/cm^2) in a flow device developed in the Dai laboratory for 24 hours. Conditioned medium (CM) and isolated ECM were collected. Enzyme-linked immunosorbent assays (ELISAs) for bFGF and EGF, as two selected factors, were performed on CM and isolated ECM. *Dissociated Sub-ventricular Zone (SVZ) Culture & Analysis* –Cells isolated from the SVZ of adult mice were expanded as neurospheres. Primary neurospheres were seeded (1.8x10^4 cells cm^2) on poly-L-ornithine coated glass in control (not EC-conditioned) or experimental (flow or static) medium supplemented with 5 ng/ml of bFGF and EGF. Viability was assessed by LIVE/DEAD. Progenitor phenotype was assessed using the neural cell-forming colony (NCFC) assay and immunostaining. p-value < 0.05 was considered significant.

Results: EC-produced bFGF and EGF increased with time in both static and flow EC-CM, but greater increases were observed in flow medium, peaking at 127 and 70 pg/10^5 cells respectively. Significant differences in bFGF and EGF sequestration in the flow-ECM were observed as early as 5 hours, reaching 1.8-fold for bFGF and 2.0-fold for EGF by 12 hours. Flow and static EC-CM supported greater viability compared to non-conditioned controls. The flow-CM spontaneously generated neurospheres, while the cells in static-CM remained adherent. Cell isolated from the SVZ were pre-conditioned in EC-CM (flow or static) for the NCFC assay. Pre-conditioned cells from EC-CM formed larger and more numerous colonies with the flow-CM group generating the largest and most numerous colonies. Colonies great than 1 mm were selected, dissociated, and are being evaluated by immunostaining to distinguish NSC from NPC derived colonies. Furthermore, cell response to the EC-ECM is underway. Both EC-ECM support cell attachment but differences in morphology are evident by day 3.

Conclusion: EC secretion is regulated through local hemodynamics. EGF and bFGF are known to be influential in NSC fate, and thus were selected as two key factors to represent the differences existing in
the static v. dynamic culture. Traditional, static models may be unrepresentative of the native *in vivo* microenvironment. Inclusion of flow-mediated changes may further enhance our understanding of the neurovascular niche.

F-2053

CHARACTERIZATION OF THE MICRORNA EXPRESSION PROFILES OF IPS CELLS AND THEIR DERIVATIVES BY MASSIVELY PARALLEL SEQUENCING

Christian Kannemeier[1], Elisabeth A. Enghofer[1], Gavin Meredith[2], Jason Myers[2], Brad Swanson[1]

[1]Cellular Dynamics International, Madison, WI, United States, [2]Life Technologies, South San Francisco, CA, United States

MicroRNAs (miRNAs) have been described as
master regulators of gene expression and a wide variety of miRNAs have been
implicated in the maintenance of cell pluripotency and differentiation. The
current study was designed to examine the stability of miRNA expression profiles
of induced pluripotent stem cells (iPSCs) derived from multiple donors and cultured
in different cell culture media. In
addition, the miRNA expression profiles of two iPSC derivatives, cardiomyocytes
and neurons, were compared across lots and over time in culture. iPSCs from three different donors were grown in either TeSR or E8 medium for at least 5 passages. Cells were harvested, miRNA
was isolated, and the samples were subjected to a miRNA sequencing protocol using
next generation sequencing techniques. After
sequencing, the number of obtained reads from each miRNA was quantified and

normalized. From these data, a miRNA expression profile for each sample was created. When the miRNA expression profile of the same iPS cell line was compared after it was grown in TeSR or E8 medium, no major differences were detected. This shows that the two culturing media are consistent in their impact on the miRNA expression profile of iPSCs. To investigate the dynamics of the miRNA expression profile of iPSC-derived cardiomyocytes and neurons, three lots of iCell Cardiomyocytes and three lots of iCell Neurons were thawed, plated and maintained in culture in standard maintenance medium for up to 3 weeks. After 7, 14, and 21 days (iCell Neurons) or 3,
7 and 14 days (iCell Cardiomyocytes), cells were harvested and subjected to the same miRNA sequencing workflow as described above. The miRNA expression profile of iCell Neurons was consistent between lots and remained unchanged during time in culture. Similarly, the miRNA profile of iCell Cardiomyocytes was consistent between lots.
Interestingly, a systematic miRNA profile shift was observed in the iCell Cardiomyocytes during the first week of culture. Specifically, more than half of all miRNAs present showed a greater than two-fold difference in expression from day 3 to day 7, but then remained stable after 7 days in culture. These data correlate with the observation that cryopreserved cardiomyocytes require several days after thawing and plating to form a stable, contracting syncytial monolayer. In conclusion, massively parallel sequencing of miRNAs revealed that iPSCs can be cultured reproducibly in either TeSR or E8 medium, and that iPSC-derived cardiomyocytes and neurons exhibit lot-to-lot consistency and stability over time in culture.

F-2054

OPTIMIZATION OF GROWTH MODELS FOR PLURIPOTENT STEM CELLS

Kevin G. Chen[1], Barbara S. Mallon[1], Kory R. Johnson[1], Rebecca S. Hamilton[1], Ronald D.G. McKay[2], **Pamela G. Robey**[3]

[1]NINDS/NIH/DHHS, Bethesda, MD, United States, [2]Lieber Institute for Brain Development, Baltimore, MD, United States, [3]NIDCR/NIH/DHHS, Bethesda, MD, United States

Human pluripotent stem cells (hPSCs) have two potentially attractive applications: cell replacement-based therapies and drug discovery. Both require the efficient generation of large quantities of clinical-grade stem cells that are free from harmful genomic alterations. The colony-type culture methods currently employed often result in low cell yields and unavoidably heterogeneous cell populations. We have recently developed a novel culture method involving non-colony type monolayer (NCM) growth. This method produces more homogeneous hPSCs than the colony-type culture methods and results in 3- to 4-fold increase in hPSC numbers in 3- to 4-days, comparable to that described in bioreactor-based suspension culture conditions. However, the NCM method requires the use of the ROCK inhibitor, Y-27632, at 10 microM to enhance the initial 24-hour single-cell plating efficiency (SCPE). In this report, we have tested the effects of various small molecules that interact with ROCK signaling pathways and extracellular matrices on both SCPE and NCM growth of hPSCs. We found that Y-39983 (ROCK I inhibitor), phenylbenzodioxane (ROCK II inhibitor), and thiazovivin (a novel ROCK inhibitor) can also modulate the SCPE and promote NCM growth at concentrations between 1 and 20 microM, similar to Y-27632. Notably, the ROCK I inhibitor appears to show cytotoxicity at 2 microM, implicating a more specific interaction than other molecules. Interestingly, a recombinant human laminin isoform (LM-521) alone, without the presence of ROCK inhibitors, supports both SCPE and the NCM culture under xeno-free conditions. These results suggest that we can generate clinically relevant quantities of homogeneous hPSCs under diverse NCM conditions. Furthermore, based on the influence of these factors on cell-cell interactions and signaling pathways in various NCM growth models, we provide new insights into concepts, strategies and solutions for production of clinical-grade hPSCs and stem cell precursors, which are the initial and pivotal steps needed for future clinical applications.

F-2055

EVOLUTION OF HUMAN PLURIPOTENT STEM CELL NUCLEAR PLASTICITY INDUCED BY GEOMETRICAL CONSTRAINS

Eleonora Grespan[1], Giovanni Giobbe[1], Florent Badique[2], Karine Anselme[2], Juergen Ruehe[3], **Nicola Elvassore**[1]
[1]Dept. of Industrial Engineering, padova, Italy, [2]Institut de Science des Materiaux de Mulhouse, Mulhouse, France, [3]University of Freiburg, Department of Microsystems Engineering, Freiburg, Germany

Nuclear shape and structure is related with cell function during developmental, physiological and pathological modifications. For instance, cancer and undifferentiated cells are associated to high nuclear deformability. Investigation of nuclear plasticity was performed to study the nucleus mechanotransduction and the correlation between nuclear morphology and diseases. However the underlying mechanisms of nuclear plasticity are still unknown. Nuclear deformability studies during development could represent an effective model to evaluate transitions in nuclear plasticity and to elucidate the triggering mechanisms. In this perspective, we aim at investigating the nuclear plasticity in human pluripotent stem cells (hPSC) from pluripotency to differentiation.
Nuclear deformability was investigated by inducing nuclear deformation through cell geometrical constrains imposed by micro-patterned substrates. Both human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC) shows high nuclear deformability, comparable to those of cancer cells (SaOs-2 osteosarcoma cells), while maintaining their pluripotency. Interestingly, cord blood mesenchymal stem cells show a degree of deformability in-between of pluripotent and somatic cells.
Nuclear plasticity were investigated along differentiated into the three germ layers and their specification was analyzed at day 4 by expression of brachyury (BraT) for mesoderm, alpha-fetoprotein (AFP) for endoderm, and beta-III tubulin (β-IIITub) for ectoderm. Nuclear behavior in early differentiation stage show that a high level of nuclear plasticity is maintained during early mesoderm and endoderm differentiation, whereas early ectoderm differentiation implies an increase in nuclear stiffness, with nuclei that emerge from the micropillars becoming round shaped. SaOs-2 osteosarcoma cells (high nuclear deformability) and fibroblasts (undeformable nuclei on micropatterned substrates) were used as positive and negative controls, respectively.
We showed that hPSC display high nuclear plasticity, which could be lost during differentiation. In perspective, hPSC can be a useful model to study nuclear plasticity transitions that are of paramount importance in physiological and pathophysiological problems, and to understand the mechanisms that control nuclear shape.

F-2056

DEVELOPMENT OF A NOVEL SYSTEM TO STUDY PROTEIN FUNCTION IN MAMMALIAN STEM CELLS

Ran Brosh, Ihor R. Lemischka
Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY, United States

Study of genes and pathways involved in regulating self-renewal and pluripotency in mammalian stem cells is commonly performed by loss-of-function strategies utilizing RNA interference. In some cases, these techniques are combined with genetic complementation strategies that allow increased specifity and modularity. We are developing a novel strategy to study gene function, specifically adapted for use in mammalian stem cells. This strategy features shRNA-based knockdown of an endogenous gene-of-interest, complemented by expression of a degradable protein-of-interest. Our system imports key components of the plant hormone-induced degradation system into mammalian cells. A plant-specific hormone receptor is expressed, which, upon binding its cognate media-supplemented hormone, recruits an ubiquitin ligase complex to any target protein that is marked by a specific Degron domain, thereby promoting its degradation. Our system is designed as a single lentiviral vector harboring multiple elements, including an U6 promoter-driven shRNA cassette and a PGK promoter driving the expression of 3 protein coding sequences (separated by 2A peptides): a codon-optimized plant hormone-receptor; an optimized Degron-fused target gene; and a selection marker. This design offers efficient knockdown of an endogenous gene-of-interest while complementing it with a degradable exogenous version. Treatment with the respective plant hormone induces rapid (~1h), titratable and reversible degradation. Importantly, by utilizing different sets of plant hormones,

hormone-receptors and Degron sequences, our system will offer combinatorial studies of protein function that are otherwise impossible. This is expected to enable a wave of quantitative and integrative analyses of protein function and protein networks in diverse stem cell types.

F-2057

DEVELOPMENT OF IN VIVO RHESUS MACAQUE AUTOLOGOUS IPSC TERATOMA AND BONE TISSUE DIFFERENTIATION MODELS: IMPORTANT STEPS TOWARDS CLINICAL SAFETY AND EFFICACY OF STEM CELL THERAPIES

So Gun Hong[1], Thomas Winkler[1], Chuanfeng Wu[1], Sergei A. Kuznetsov[2], Robert E. Donahue[1], Mark E. Metzger[1], Vicky Guo[1], Stefania Pittaluga[3], Alina Nicolae[3], Tina Kilts[2], Li Li[2], Pamela G. Robey[2], Cynthia E. Dunbar[1]
[1]Hematology Branch, NHLBI/NIH, Bethesda, MD, United States, [2]Craniofacial and Skeletal Diseases Branch, NIDCR/NIH, Bethesda, MD, United States, [3]Laboratory of Pathology, Hematopathology section, NCI/NIH, Bethesda, MD, United States

Transgene-free rhesus macaque iPSCs (RhiPSCs) were derived using the Cre-excisable STEMCCA vector, leaving a short non-expressed vector DNA tag for subsequent tracking studies. RhiPSCs, adapted to culture on Matrigel or the chemically-defined synthetic surface SynthemaxII, retained their pluripotent phenotype. We used the RhiPSCs first to develop a clinically-relevant autologous teratoma model in the presence of an intact immune system, and second to evaluate the potential of clinical use of RhiPSC derived mesodermal stromal-like cells (RhiPS-MSCs) for *in vivo* bone regeneration. RhiPS-MSCs expressed typical surface markers (CD44, CD73, CD90, CD105, and CD166) and could further differentiate to the osteogenic lineage *in vitro*.
$5X10^5$- $2X10^6$ cells from three independent RhiPSC clones grown on Matrigel were subcutaneously injected into both the donor autologous rhesus monkey and immunocompromised NSG mice. Instead of a xenogenic scaffold (e.g. Matrigel), an autologous plasma clot method was developed to support teratoma formation. Simultaneously, autologous RhiPS-MSCs ($2X10^6$) mixed with hydroxyaptite/tricalcium phophate (HA/TCP) ceramic particles were subcutaneously implanted to investigate *in vivo* bone formation. After 4-6 weeks, all RhiPSCs injected into NSG mice formed mature teratomas. In contrast, only one out of six autologous RhiPSC implants had formed a small mass (~4mm) in the macaque. Histologically, the mass resembled homogenous fibrotic tissue with no typical teratoma structures. PCR for the vector LTR confirmed RhiPSC origin. In contrast, simultaneously implanted RhiPS-MSCs in HA/TCP formed unequivocal bone tissue in the autologous monkey, without any evidence of teratoma formation or an inflammatory reaction. Although the RhiPSCs were extensively washed prior to the implantation, we could not rule out that an immune or inflammatory reaction to residual Matrigel contributed to the failure of RhiPSCs to form teratomas. We therefore repeated the experiment using cells grown on the SynthemaxII, and also increased the maximal number of injected cells up to $1X10^7$. Again, $5X10^5$ RhiPSCs did not form detectable masses by 18 weeks. Sites injected with $1X10^7$ cells exhibited an early tumor growth (~9mm) that halted thereafter. Tumors were harvested at 2, 5, and 10 weeks. Immature germ layer structures were found in tumors obtained after 2 and 5 weeks. There was a significant lymphocytic and eosinophilic infiltration in the tumor and the surrounding tissue, composed of CD3+, CD4+, CD20+, and a few CD56+ cells. At 10 weeks, for the first time, unequivocal mature teratoma structures including neural, mesenchymal, and glandular components were seen. However, less robust differentiation and proliferation was seen compared to teratomas derived from the same cells injected into NSG mice.
In summary, we have for the first time succeeded in developing an autologous pre-clinical iPSC transplantation model in an immunocompetent large animal with clinical relevance, demonstrating both autologous teratoma and differentiated tissue engraftment. However, slow growth of teratoma and inflammatory infiltrates suggest an immune reaction of host cells against undifferentiated iPSCs but not iPSC-derived differentiated tissues. To our knowledge, this is also the first report of autologous *in vivo* bone formation from iPSCs in non-mouse animal models.Studies investigating the potential immunogenicity of iPSC are ongoing and will be presented at the meeting.

F-2058

COMPARISON OF TWO DIFFERENT DEFINED PROTEIN DELIVERY SYSTEMS FOR STEM CELL MANIPULATING (CELL-PENETRATING PEPTIDE VS. STREPTOLYSIN O)

Junghyun Jo, Soomin Hong, Sook-Young Yoon, Dong Ryul Lee
Biomedical Science, CHA University, Seoul, Korea, Republic of

Introduction:
Delivery of defined factors can induce alteration of cellular properties and could be a useful tool in the evolving field of cell research and, especially, for the clinical application of stem cells. Intracellular protein delivery system is most safe and useful clinical approach among various others because of conducting transient uptake and regulation of gene expression without genetic modification. Recently, several systems such as cell-penetrating peptide (CPP)- and streptolysin O (SLO)-mediated are introduced as useful tools for the delivery of biologically active proteins. Our group reported that CPP (7x arginine, R7)-conjugated protein have been delivered into human mesenchymal stem cells (hMSCs) efficiently and operated as biological active protein (Jo et al., Stem Cells, 2012). Also, SLO as pore-forming bacterial toxin have been widely used to bring exogenous protein into living cells (Cho et al., Blood, 2010). However, the efficiency between two intercellular protein delivery systems has not been compared until now. So, the aim of this study is to compare firstly the relative amount of protein delivery into hMSCs, their cytotoxicity and biological function between two different systems (CPP- and SLO-mediated) in order to provide a more useful tool for exogenous defined protein delivery in manipulating stem cells.
Materials and Methods: For these comparisons, two different proteins were used for the study. One is green flourescence protein (GFP) that is visualized easily using a confocal microscope, and used for evaluating protein transduction efficiency. The other is estrogen-related receptor β (ESRRB) that is known to interact with pluripotency-related factors. The delivery of ESRRB protein was to perform for analyzing cytotoxicity and biological activity. These proteins were purified in native form with / without R7. CPP-GFP and CPP-ESRRB proteins were treated to hMSCs derived from bone marrow (BM) and testis (TS). GFP and ESRRB proteins were delivered into hMSCs by SLO mediated system. Protein transduction efficiency and quantification was analyzed by confocal microscope and Western Blot, and cytotoxicity and apoptosis of both protein delivery systems were also compared. In addition, we examined up-regulation of the pluripotency-related genes using qRT-PCR to analyze biological activity of ESRRB protein.
Results:
The intracellular uptake efficiency of CPP-GFP was higher than that of SLO mediated GFP in both BM- and TS-hMSCs through confocal microscopy image. The delivered GFP and ESRRB protein quantity was reconfirmed by Western Blot. Additionally, in groups mediated with CPP conjugated protein, viability of hMSCs was higher and apoptosis rate was lower than SLO mediated protein delivery group. qRT-PCR showed that the expression level of *OCT4*, *SOX2*, and *NANOG* were increased more in CPP-ESRRB-delivered hMSCs than those in SLO-ESRRB-delivered ones.
Conclusion:
We compared two different protein delivery systems (CPP- and SLO-mediated) and resulted that CPP-mediated system has a higher efficiency for intracellular delivery

and a lower cytotoxicity than SLO-mediated. Also, delivered ESRRB protein into hMSCs up-regulates expression level of the pluripotency-related genes, and their biological activity may be confirmed. Therefore, CPP-mediated protein delivery system is a highly suitable tool for exogenous defined protein delivery for manipulating stem cells, especially, in clinical applications of cell-based therapy.

F-2061

PULSED FOCUSED ULTRASOUND (PFUS) IS A NONDESTRUCTIVE MODALITY THAT PROVIDES TUNABLE CONTROL OVER TARGETED HOMING OF MESENCHYMAL STEM CELLS (MSC) AND ENDOTHELIAL PRECURSOR CELLS (EPC)

Scott R. Burks, Ali Ziadloo, Saejeong J. Kim, Ben A. Nguyen, Joseph A. Frank
Radiology and Imaging Sciences, National Institutes of Health, Bethesda, MD, United States

Introduction: While stem cell (SC) therapies have shown promise, cell homing to pathological loci following systemic infusion is extremely inefficient (<1-3% injected dose). Circulating stem cells home by tethering to activated endothelium and actively transmigrating into the parenchyma in response to cytokine/chemokine and trophic factor (CCTF) gradients. pFUS treatment to skeletal muscle drives a transient and local molecular biological response, likely through mechanotransduction, which mimics transient inflammatory profiles that can attract circulating MSC. Our objectives were to further characterize, histologically and with molecular biological assays, the nondestructive nature of pFUS and whether enhanced homing permeability and retention (EHPR) of MSC and other stem cell types could be targeted by pFUS. We also investigated if the EHPR effect could be fine-tuned using pFUS (i.e. whether additional sonication/injection treatments had cumulative effects on cell homing).
Methods: Murine hamstring skeletal muscle was sonicated (1 MHz, 40 W, 5% duty cycle, 1 Hz repetition frequency) without microbubbles. For cell injections, human MSC or CD34+/CD133+ EPCs were iv injected 2 hr after pFUS. Mice were treated over the course of 3 days with combinations of pFUS and SC injections spaced 24 hrs apart. Tissues were harvested for physiological, histological, and molecular analyses.
Results: Mice were given single or multiple treatments (every 24 hr for 3 days) of pFUS alone (no cells). Both exposure courses were nondestructive to skeletal muscle and did not induce hemorrhage, necrosis, or apoptosis. Mast cell proliferation or degranulation was not detected and pFUS did not activate Pax7, a transcription factor necessary for muscle repair. pFUS elicited a small, predominately M2 anti-inflammatory macrophage response. However, both single and multiple pFUS treatments created short-lived local increase in several CCTFs and cell adhesion molecules (CAMs) that mediate stem cell homing. Accordingly, both MSC and EPC exhibited an EHPR effect in greater numbers following a single pFUS treatment. pFUS targeting of cells (MSC or EPC) is also tunable. When pFUS/cell treatments were repeated daily over 3 days, the EHPR effects of the SCs were significantly increased compared to muscle receiving only a single treatment of pFUS/cells (Fig 1). Furthermore, if pFUS and SC are given on the 1st day, but only iv cells on days 2 and 3 (no pFUS), additional increases in homing were not observed, suggesting additional pFUS treatments are required to achieve a compounded EHPR effect.
Discussion: pFUS can noninvasively and nondestructively direct SC migration in vivo. Presumably through mechanotransduction, pFUS drives a local, transient, and generic biological response creating a “molecular zip code” that can be capitalized on to target delivery of multiple SC types to different tissues. This molecular zip code has increased CCTF and CAMs such that the number of cells homing to targeted tissue can be increased through repeated sonications coupled with SC injection. This approach of modifying local host tissue rather than the cell product creates a readily translatable approach to improve efficiency and efficacy of many cellular therapies.

F-2062

MICROFLUIDIC CELL SEPARATION FOR TAG-FREE ISOLATION OF STEM CELL SUBSETS

Shashi Murthy
Northeastern University, Boston, MA, United States

The most widely employed technique for sorting selected subsets of cells in stem cell biology is fluorescence activated cell sorting (FACS). While highly versatile in its capability to isolate cells against broad panels of markers, this technique requires considerable time (at least 2 hours from the start of incubation with fluorophores to completion of sorting) and access to expensive instrumentation typically found in core facilities. The closely related technique of magnet-activated cell sorting (MACS) is not as constrained with respect to instrumentation complexity and cost but still requires a similar level of processing time. We have designed microfluidic cell separation systems that employ affinity based cell capture to isolate stem and progenitor cells from a broad range of sources, including blood and digested tissue. These systems work by using degradable hydrogel coatings within chips that can be functionalized with cell capture molecules. Following injection of the sample through the chips, a simple injection step with a chelator solution elutes out the desired cell type with high purity (>90%). Separation against one or two markers can be accomplished in 30-60 min without the need for any pre-processing labeling of the sample with fluorescent tags or magnetic beads. Specific examples of the process will be highlighted including antibody mediated enrichment of CD34+/Flk1+ cells from untreated human blood (which are of interest in reprogramming), CD34+ and Lgr5+ stem cell isolation from skin and intestine, respectively, and lectin-mediated isolation of pluripotent cell subsets. These microfluidic devices can be scaled down to process as little as 200-300 microliters of cell suspension, or scaled up to run tens to hundreds of milliliters using massively parallel arrays of chips. The cell separation process is entirely passive and does not require any instrumentation beyond a pump to drive fluid flow. The significance of this approach lies in its simplicity and scalability, which may be useful considerations in areas such as reprogramming, where well-defined cell subsets must be routinely obtained in quantity, or mechanistic studies where for example small quantities of defined subsets must be obtained reproducibly and rapidly to form co-cultures and/or incorporate mechanical stimulation.

F-2063

rBC2LCN, A POWERFUL LECTIN PROBE FOR HUMAN PLURIPOTENT STEM CELLS

Yasuko Onuma, Hiroaki Tateno, Jun Hirabayashi, Makoto Asashima, Yuzuru Ito
Research Center for Stem Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan

Both positive and negative selections of pluripotent stem cells are key steps for the practical applications, such as to reduce risk of teratoma formation in transplantation therapy and to maintain homogeneous pluripotent cell populations in culture. Recent approaches have focused on the cell surface markers, which are specifically expressed on human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), for cell-sorting by flow cytometry or magnetic cell separation. The typical pluripotent stem cell markers are cell surface glycans and antibodies, such as Stage-specific embryonic antigens -3 and -4 (SSEA-3, SSEA-4), SSEA-5, Tumor rejection antigens-1-60 and -1-81 (TRA-1-60, TRA-1-81). However, a convenient, nontoxic, inexpensive and specific probe for hiPSCs is required strongly for medical and industrial application. Here we show a recombinant lectin probe, rBC2LCN, is a new tool for fluorescent dye-based imaging and flow cytometry analysis of the pluripotent stem cells, as an alternative to conventional antibodies. Fluorescent dye-conjugated rBC2LCN allowed us to visualize colonies of both hESCs and hiPSCs with easy stain steps. Fluorescent dye-conjugated rBC2LCN also recognized live pluripotent stem cells, and separated them from mixed cell population by flow cytometry. These results suggest that rBC2LCN is a useful tool for evaluation of hESCs and hiPSCs and is hopeful for improvement of cell sorting efficiency in processes of medical and industrial application of pluripotent stem cells.

F-2064

OPTIMIZATION OF HUMAN PLURIPOTENT STEM CELL SUSPENSION CULTURES IN CONTROLLED, STIRRED BIOREACTORS

Ruth Olmer, Ulrich Martin, Robert Zweigerdt
LEBAO, Hannover Medical School, Hannover, Germany

Therapeutic and industrial applications of pluripotent stem cells and their derivatives requires large cell quantities generated in defined conditions. Taking advantage of the defined culture medium mTeSR (STEMCELL Technologies) we have translated single cell inoculated suspension cultures of human pluripotent stem cells (hPSCs; including human induced pluripotent stem cells (hiPS) and human embryonic stem cells (hESC)) to stirred tank bioreactors. These systems are widely used in biopharmaceutical industry, allow straight forward scale up and detailed online monitoring of key process parameters. To ensure minimum medium consumption, but in parallel functional integration of all probes mandatory for process monitoring i.e. for pO_2 and pH, experiments were performed in 100 ml culture volume in a "mini reactor platform" consisting of 4 independently controlled vessels (DASGIP). By establishing defined parameters for tightly controlled cell inoculation and aggregate formation up to 2 x 10^8 hiPSCs / 100 ml were generated in a single process run in 7 days. Expression of pluripotency markers and cells ability to differentiate into derivates of all three germ layers in vitro was maintained, underlining practical utility of this new process. Yet, however, only linear (rather than exponential) growth rates were achieved in bioreactors and a relative low cell density of up to ~2 x 10^6 hiPSCs / ml was obtained, suggesting a great potential for further improvements. Towards this end we will present new strategies and data on process optimization, leaping forward towards the controlled mass expansion of hPSCs and their derivatives.

Funding sources: REBIRTH Cluster of Excellence (DFG EXC62), BIOSCENT (European Union, European Atomic Energy Community, Seventh Framework Program [FP7/2007-2012] under grant agreement n° 214539), StemBANCC (Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115439, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme [FP7/2007-2013] and EFPIA companies' in kind contribution), STEMCELL Technologies Inc. (Vancouver, Canada), DASGIP Information and Process Technology GmbH, (Jülich, Germany)

F-2071

STRUCTURE-BASED INVESTIGATION OF WNT SIGNALING MODULATORS

Jie Zheng
Structural Biology, St Jude Children's Res Hospital, Memphis, TN, United States

The canonical Wnt signaling pathway plays critical regulatory roles in development, stem cells and cancer. Aiming to further understand the stepwise signaling cascade in the Wnt pathway and to develop potential novel pharmaceutical agents that interfere with aberrant Wnt signaling, we have taken a systems biology approach by developing small molecules that modulate the protein-protein interaction at different signaling steps in the Wnt pathway. While Wnt inhibitors have therapeutic potential in anti-cancer therapy, Wnt signaling activators may be useful in maintaining stem cell pluripotency, regulating cell regeneration. Based on the concept of "inhibitor of an inhibitor is an activator", we hypothesize that compounds that can interrupt the functions of Wnt antagonists would function as Wnt activators. Initial hits for the inhibitors of Wnt antagonists sFRPs and DKKs have been obtained. The functions of these activators are being evaluated. We have also developed small molecules that can reverse the DKK antagonistic effect by specifically binding to the DKK-binding cavity of the third β-propeller domain of LRP5/6 and block the interaction.

F-2072

STABLE FEEDER- AND XENO-FREE SURFACES FOR LONG-TERM GROWTH OF UNDIFFERENTIATED HUMAN EMBRYONIC STEM CELLS

Amanda DiIenno, Anna M. Coclite, Jeffrey R. Millman, Jit Hin Tan, Clark K. Colton, Karen K. Gleason
Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, United States

Stem cells hold enormous potential for application in regenerative medicine and tissue engineering. Human embryonic stem cells (hESC) are difficult to culture and grow while maintaining an undifferentiated state. Currently used techniques of culture on mouse embryonic fibroblast feeder layers or on a layer of Matrigel introduce xenogeneic proteins of unknown composition. Approaches for producing feeder-free culture surfaces composed of well defined human components are in their infancy. Most rely on physical adsorption of proteins, which provides limited long-term stability of the protein layer. Silicone rubber (SiR) membranes were selected as the culture substrate for this study because recent reports show that control of oxygen level at reduced levels can aid directed differentiation. However, hESC do not attach and grow on uncoated SiR. Here we describe a compositionally defined matrix that supports hESC expansion over more than five passages. It consists of a cross-linked hydrogel with carboxylic functional groups, produced by initiated Chemical Vapor Deposition (iCVD), which is deposited on SiR. This is the first report of iCVD coatings used for hESC growth. The advantages of this approach are that (1) it is possible to coat SiR without modifying its oxygen permeability; (2) iCVD allows easy copolymerization with monomers bearing useful functional groups such as carboxylic groups that can be easily functionalized with peptides and proteins; (3) iCVD-prepared hydrogels can have a tunable cross-linking density that makes them stable under sterilization conditions and long term storage; and (4) iCVD allows easy tuning of the functional group density on the surface which directly relates to the density of peptides or proteins covalently bonded on the surface. Changes in the vapor feed ratio of the monomers used in the iCVD process allowed variation of the density of -COOH groups on the surface. The -COOH groups were functionalized by reaction with -NH2 groups of proteins in order to covalently bond proteins to the surface. The formation of amidic (-CONH) groups was detected by FT-IR. The surface density of bound protein (for this study it was fibronectin) increased with increasing surface density of COOH groups. The density of protein on the surface influenced cell attachment and proliferation. Cell attachment was monitored with DAPI staining, and pluripotency was monitored with the transcription factor OCT4. For intermediate densities of COOH groups, the cells formed a uniform confluent monolayer on the surface. OCT4 expression was seen in the majority of the seeded cells. When the density of COOH groups (and consequently of protein) on the surface was the highest, the cells formed agglomerates but did not form a uniform monolayer. When the density of the COOH groups on the surface was the lowest, the cells formed a uniform monolayer but did not form a confluent surface after seven days of culture. The loss of confluency and monolayer formation resulted in spontaneous differentiation. Through more than 5 passages, hES cells seeded on the coated surfaces displayed a normal karyotype, retained pluripotency, and formed teratomas after implantation into SCID/Beige mice. The stable, xeno-free synthetic approach for hESC culture described here is important for future scale-up of hESC production.

F-2073

HEMATOPOIETIC REPORTER AND KNOCKDOWN HESC LINES GENERATED BY SITE-DIRECTED TRANSGENE TARGETING

Amita Tiyaboonchai, Helen Mac, Jason A. Mills, Razveen Shamsedeen, Prasuna Paluru, Deborah L. French, Paul Gadue
Children's Hospital of Philadelphia, Philadelphia, PA, United States

Human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells provide a powerful model system for the study of early human development, disease modeling and gene correction. Stable transgene expression in human ES/iPS cells has been difficult due to gene silencing upon cellular differentiation and potential mutagenesis due to random integration of the targeting construct. Improved gene expression systems in human ES/iPS cells are needed for both the study of basic biology and for gene correction. Gene targeting to a safe harbor locus overcomes these difficulties, as shown by zinc finger nuclease (ZFN)-mediated targeting of transgenes into the AAVS1 safe harbor locus in human ES/iPS cells. Instead of using electroporation which is very toxic to cells, we used a lipid

transfection reagent to target the vector and ZFNs to the AAVS1 locus with a targeting efficiency of ~90%. By using this modified transgene targeting system, we have generated hematopoietic reporter and stable knockdown ES cell lines. The hematopoietic reporter line was created using an AAVS1 targeting construct containing ~2kb of the human CD43 promoter and GFP. Hematopoietic differentiation of this reporter line shows that GFP expression mirrors endogenous CD43 expression on the surface of cells as shown by flow cytometry. By replacing GFP with a gene of interest, this system can be used for transgene correction of disease-specific iPSC lines in hematopoietic disease modeling studies. The gene knock-down ES cell line was created using an AAVS1 targeting construct containing the chicken actin promoter driving the expression of short hairpin RNAs in a mirRNA backbone. As a proof of principle, we chose to knockdown PU.1 because of its role as a master regulator of myeloid cell development. By creating a cell line containing a construct with two different pairs of PU.1 specific hairpin RNAs, we show ~85% knockdown of PU.1 and a complete loss of myeloid cell development during hematopoietic differentiation. These data agree with the observed phenotype in PU.1 knockout mice and demonstrate conserved PU.1 function. In both the reporter and knockdown cell lines, gene expression was not silenced and remained stable during hematopoietic differentiation. This technology has potential for a broad range of applications including the generation of cell type specific reporters, gene knock-down studies, and transgene correction of iPSC lines for disease modeling.

F-2074

APPLICATION OF CHEMICAL BIOIMAGING PROBES FOR STEM CELL STUDY

Nam-Young KANG[1], Sung-Jin Park[1], Seong-Wook Yun[1], Yogeswari Chandran[1], Jin-Mi Kim[2], Zhi Yen Jow[1], Young-Tae Chang[2]

[1]Singapore Bioimaging Consortium, Singapore, Singapore, [2]Department of Chemistry, National University of Singapore, Singapore, Singapore

Directed differentiation of stem cells and reprogramming of somatic cells into stem cells are the key issues in stem cell research. The most demanding requisites in basic research and clinical applications of stem cells is to develop tools and methodologies for detecting and isolating specific type of stem cells at different stage of differentiation and reprogramming. Bioimaging which employs highly sophisticated imaging probes is becoming an emerging and rapidly growing field in biomedicine. A conventional strategy for fluorescent probe development is to conjugate fluorophores to known reactive molecules. However, the added fluorophores can change the activity and property of the probes making it inapplicable for use in complex biological systems. Therefore, the development of more convenient and safe fluorescence methods to detect specific celltype is a highly unmet need for both basic research and clinical applications of stem cells. We have developed a Diversity-Oriented Fluorescent Library (DOFL) composed of more than 10,000 intrinsically fluorescent small molecules by combinatorial chemistry, which has proven to be very useful in addressing problems in chemical biology and pharmaceutical field for the discovery of biologically active molecules. We have previously developed sensors and imaging probes for DNA, RNA, GTP, albumin, glutathione, heparin and beta amyloid to name a few. As an expansion of high throughput DOFL screening, we established live cell microscope image-based and flow-cytometry based high content screening systems to develop stem cell type- and differentiation stage-specific colorful chemical imaging probes. Detail examples and applications of the stem cell probes will be further discussed.

F-2081

TALEN-MEDIATED TARGETING OF THE BETA-GLOBIN GENE IN HUMAN CD34+ CELLS FOR THE TREATMENT OF SICKLE CELL DISEASE

Adam J. Hartigan[1], Richard Voit[2], Matthew Porteus[2], Amy J. Wagers[1]

[1]Developmental and Stem Cell Biology, Joslin Diabetes Center and Department of Stem Cell and Regenerative Biology, Harvard University, HHMI and Harvard Stem Cell Institute, Boston, MA, United States, [2]Department of Pediatrics, Stanford University, Palo Alto, CA, United States

Sickle cell disease (SCD) affects millions of people worldwide and occurs in 1 in 500 African Americans in the United States. Current therapeutic approaches directed against SCD aim to limit its symptoms. However, individuals suffering from the disease have ongoing complications and a shortened life expectancy. The only known cure for SCD is an allogeneic bone marrow transplantation, which is limited in its utility by a lack of suitable donors and the high incidence of morbidity and mortality following transplantation. Using human hematopoietic stem and progenitor cells (CD34+ cells), our aim is to correct the mutated beta-globin gene responsible for SCD by transfecting these cells with beta-globin-specific TAL effector nucleases (TALENs). This approach allows for the use of corrected autologous CD34+ cells during transplantation, thereby eliminating the need for donor CD34+ cells and decreasing the risks associated with allogeneic bone marrow transplantation. Our studies have shown that CD34+ cells can be efficiently transfected with beta-globin-specific TALENs and a donor plasmid that contains GFP under the control of the endogenous beta-globin promoter. Initial data indicates a gene-targeting rate nearing one percent in human CD34+ cells. Targeting rates can be doubled in CD34+ cells using O6-methylguanine-DNA-methyltransferase, a drug resistant gene within the donor plasmid. These ongoing experiments will generate the preclinical data necessary for a potentially new SCD therapy.

F-2082

STEM CELL DYNAMICS IN THEIR NATIVE 3D NICHE: IMAGING THE AGING MOUSE SUBVENTRICULAR ZONE

Chris S. Bjornsson[1], Eric C. Wait[2], Mark R. Winter[2], Yue Wang[1], Erzsebet Kokovay[3], Andrew R. Cohen[2], Sally Temple[1]

[1]Neural Stem Cell Institute, Rensselaer, NY, United States, [2]Department of Electrical & Computer Engineering, Drexel University, Philadelphia, PA, United States, [3]Department of Cellular & Structural Biology, University of Texas Health Science Center, San Antonio, TX, United States

The mammalian subventricular zone (SVZ) generates fewer mature neurons with age. In order to investigate the intrinsic and extrinsic cues responsible for this decline, many studies focus on lineage tracing using in vitro dissociated cultures or static time points. While the thin, planar organization of the SVZ makes it ideal for microscopic examination, long-term time-lapse images of stem cell behavior in their native, intact tissue have proven difficult to obtain. In the adult SVZ mature, fully differentiated cells intermingle with neural stem cells to create the stem cell niche. This complex architecture yields an explant preparation with unique culture requirements, especially when conducting long-term time lapse image collection with an inverted confocal microscope.

We have characterized whole mount SVZ (wmSVZ) viability after 20 hours in culture under different conditions using confocal microscopy of immunolabeled tissue. Perfusing oxygenated artificial CSF, the standard for electrophysiological recording of adult tissue slices, resulted in widespread cell death after 20h, evidenced by degenerating and disorganized GFAP+ processes and vasculature, cleaved caspase 3 expression, and pyknotic or highly blebbed nuclei. Another traditional method, culture medium formulated for filter-supported organotypic tissue explants maintained viability of cells along the outer surface, but cells deeper than 15 µm into the tissue exhibited telltale signs of degeneration. The SVZ niche is approximately 30 µm thick, with transit amplifying C cells and neuroblasts (A cells) residing deeper into the tissue, therefore ensuring the viability of these deeper cell layers is essential to derive meaningful information. To address the concern that cells further interior may be receiving insufficient oxygenation, we introduced a mixture of 95% O2/5% CO2 into the headspace above the culture media to create a standing oxygen gradient. With the appropriate media volume, deeper tissue regions remained viable for 20 hours, however in this case superficial cells closer to the fluid/air interface showed signs of damage and death.

To arrive at a compromise suitable for imaging submerged tissue on an inverted microscope, we have fabricated a custom imaging chamber that suspends the tissue above the dish bottom to permit flow of oxygenated fluid underneath. Using this approach we are able to image explanted wmSVZs from adult and aged mice for 20 hours using confocal microscopy to generate 3D time-lapse data. Neural stem cells (B cells) expressing GFP or transit amplifying cells (C cells) expressing mTomato can be observed in relation to the ependymal layer and niche vasculature, both labeled with fluorescent isolectin B4. Time-lapse multispectral 3D datasets are then analyzed using custom image analysis software that we developed called LEVER-3D. The software segments, tracks and lineages proliferat-

ing cells together with the vasculature in the 3D image sequence, and provides an intuitive interface for users to inspect results and correct any errors. LEVER-3D uses computer gaming hardware to provide interactive stereoscopic 3D visualization of the image sequence data, allowing the intimate and dynamic associations between neural stem cells and the neighboring vasculature and ependymal niche cells during cell division to be quantified, and enabling an unprecedented ability to investigate the aging SVZ.

F-2083

PROTEOMIC AND TRANSCRIPTOMIC COMPARISON OF HUMAN PLURIPOTENT STEM CELLS GROWN IN ADHESION CULTURE OR AS SUSPENSION CULTURE SPHEROIDS

Sarah A. Konze[1], Laura van Diepen[4], Anke Schröder[2], Andreas Pich[2], Ruth Olmer[3], Robert Weißmann[4], Andreas W. Kuss[4], Robert Zweigerdt[3], **Falk F. Buettner**[1]
[A]Institute for Cellular Chemistry, [B]Institute of Toxicology, [C]Leibniz Research Laboratories for Biotechnology and Artificial Organs / HTTG, [1]Hannover Medical School, Hannover, Germany, [2]Institute for Human Genetics & Institute for Genetics & Functional Genomics, Ernst-Moritz-Arndt University, Greifswald, Germany

Recent protocols enable the expansion of human pluripotent stem cells (hPSCs) in suspension culture. Assessing molecular changes of hPSCs associated with the transition from adherent to suspension culture conditions is important, as culture conditions might influence differentiation capacity and therapeutic application of these cells. In order to identify differences in gene expression associated with the transition of hPSCs from adherent to suspension culture, we used a stable isotope labeling by amino acids in cell culture (SILAC)-based proteomic approach in combination with transcriptomics. One human embryonic stem cell (hESC) line and one human induced pluripotent stem cell (hiPSC) clone were SILAC labeled with fully preserved pluripotency. We quantitatively analysed the overall proteome status at a depth of 3742 proteins and showed 99 proteins being upregulated in suspension culture and 617 being downregulated. Suspension culture-induced alterations identified by both, proteomics and transcriptomics, showed perfect concordance. Expression of histones and other factors involved in nucleosome assembly were upregulated in suspension culture as were secreted inhibitors of the canonical Wnt signalling pathway. On the other hand, factors that mediate cell-cell or cell-matrix interactions were downregulated in suspension culture. Moreover, cells grown in suspension culture spheroids appear to build their own microenvironment at the proteomic level as deduced from the observed enrichment of several secreted proteins involved in embryogenesis or belonging to the extracellular matrix, while their respective expression level remained unchanged in suspension culture spheroids compared to adherent cultures.

F-2084

USE OF SURROGATE REPORTER VECTORS TO ENRICH FOR TALEN MODIFIED CELLS

Natasha Roark, Jian-Ping Yang, Veronica Blackston, Jonathon Chesnut
Synthetic Biology R&D, Life Technologies, Carlsbad, CA, United States

The ability to effectively model human diseases is often hindered by the difficulty in altering nucleotide sequences and influencing gene expression in a targeted fashion. Engineered transcriptional activator-like effector nucleases (TALENs) have emerged as attractive tools for inducing genetic modifications as they can be designed *de novo* to target specific DNA sequences and modify DNA at that locus. Given their modular structure and specificity, TALENs allow for the control of cellular gene expression in a precise, predictable and robust manner. An impediment to the many applications involving TALENs is the ability to select or enrich for genetically modified cells, as they are generally phenotypically indistinguishable from wild types. This is further complicated by the fact that often only a small population of cells will contain the desired mutation, necessitating the screening of many clones. This difficulty is compounded in hard to culture cell types such as induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs).

For this purpose we constructed surrogate reporter vectors containing the target sequence and encoding an out of frame green florescent protein (GFP) and CD4 membrane protein. A TALEN-induced double-stranded break will be repaired by non-homologous end joining (NHEJ), resulting in a frameshift mutation that will render the GFP and CD4 in-frame in a fraction of the modified cells. This enables us to enrich for genetically modified cells using flow cytometry and antibody-coated magnetic beads, as only cells containing the TALEN modified reporter plasmids will express CD4 or GFP. Cells were co-transfected with the aforementioned reporter vector and corresponding TALEN pair and subsequently enriched for CD4 and GFP expression using magnetic beads and flow cytometry, respectively. An endonuclease-based mismatch detection assay was then used to quantify the percentage of gene modification in pre and post-enrichment samples. Preliminary findings show that antibody coated beads and GFP-based cell sorting provide approximately three-fold and four-fold enrichment, respectively. The fold enrichment is inversely proportional to the number of clones one must screen to obtain the desired mutation, i.e. with four-fold enrichment the number of clones screened can be reduced by one-fourth. This project demonstrates that utilizing a surrogate reporter vector is an effective and reliable tool enabling one to selectively enrich genetically modified cells, obviating the need to isolate a large number of clones. The results from this project have a broad range of practical applications for modeling human disease including the development of knock-in, knockout and transgenic organisms, particularly for difficult to culture cell-types such as iPSCs and ESCs.

F-2091

NANOFIBRILLAR CELLULOSE HYDROGEL SUPPORTS THREE-DIMENSIONAL CULTURE OF HUMAN PLURIPOTENT STEM CELLS

Yan-Ru Lou[1], Liisa Kanninen[1], Tytti Kuisma[1], Johanna Niklander[1], Luke Noon[3], Deborah Burks[3], Arto Urtti[2], Marjo Yliperttula[1]

[A]Division of Biopharmaceutics and Pharmacokinetics, Faculty of Pharmacy, [B]Centre for Drug Research, Faculty of Pharmacy, [1]University of Helsinki, Helsinki, Finland, [2]Laboratorio de Neuroendocrinología Molecular, Principe Felipe Centro de Investigacion, Valencia, Spain

Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) are pluripotent and capable of self-renewal. They can differentiate to all the cell types in human adult and therefore they have great potential in research and therapies. To provide high quality cells for various applications, we have developed a culture system using a novel plant-derived nanofibrillar cellulose (NFC) hydrogel. Human ESCs and iPSCs cultured in the NFC hydrogel formed three-dimensional (3D) spheroids with strong OCT4 expression. After enzymatic removal from the hydrogel, the stem cells were able to attach to 2D matrix-coated surfaces, exhibiting typical stem cell morphology and expressing the pluripotency markers OCT4 and SSEA-4 but not differentiation markers muscle actin, β-tubulin type III or HNF3B. The cells cultured in the NFC hydrogel can be differentiated into the cells of three germ layers via *in vitro* embryoid body formation and *in vivo* teratoma formation, indicating that the cells retain pluripotency in 3D culture. This culture system uses biomaterial with no animal products. It will be useful in stem cell-based drug research, cell therapy and tissue engineering.

F-2092

INTEGRATED MULTI-STAGE TISSUE ON A CHIP GENERATION FROM HUMAN PLURIPOTENT STEM CELLS

Federica Michielin, Giovanni Giobbe, Sebastian Martewicz, Stefano Giulitti, Annarosa Floreani, Nicola Elvassore
Industrial Engineering, University of Padova, Padova, Italy

Direct generation of functional tissues from human pluripotent stem cells (hPSCs) *on a chip* would provide an attractive tool for multi-parametric studies in human development and diseases. Stem cells expansion, selective germ layer commitment and mature differentiation are sequential stages for obtaining tissue-specific cells. These developmental stages require an accurate balance between extrinsic and intrinsic cell signaling through an optimal *in vitro* regulation of delivered exogenous factors and endogenous cell-secreted factors.

In this study, we derived committed germ layers and functional hepatic and cardiac cells on a chip from hPSCs through a multi-stage microfluidic-based technology. Effective and selective commitment is achieved by guiding cell niche specification in terms of exogenous and endogenous stimulations, through stage-dependent frequency optimization of microfluidic discontinuous medium delivery. Computational investigations on extrinsic signaling model reveals that frequency of medium delivery strongly affects accumulation of exogenous-endogenous factors, thus regulating transcriptional activity pattern of target genes.
Quantitative Real Time PCR analysis of pluripotency markers after 6 days of both embryonic and pluripotent stem cell culture, showed an optimal frequency of 2 cycles/day; 3-fold Oct4 expression compared to lower (1 cycle/day) and higher frequencies (4-8 cycles/day) was observed. Endoderm, mesoderm and ectoderm germ layer commitment was successfully induced on hPSCs expanded within microfluidic channels through soluble factors stimulation by using frequency of 2 cycles/day. Alpha-fetoprotein, BrachyuryT and beta3-tubulin-positive cells in the three cases respectively, resulted highly expressed and comparable with standard static conditions. Selective induction of mesoderm commitment was achieved by adjusting frequency of medium delivery (4 cycles/day) yielding to 1 fold BrachyuryT expression increase and substantial decrease of AFP and beta3-tubulin expression.
Cardiac cells on a chip were derived after 10 day of frequency-dependent multi-stage differentiation protocol, consisting on mesoderm induction (Wnt induction/stimulation), early cardiac commitment (Wnt inhibition) and functional cardiac maturation (pro-cardiac factor supply). 60% Troponin-T positive cells showed defined cardiac sarcomeric organization, spontaneous contractile activity, excitation contraction coupling up to 2 Hz and proper calcium dynamics with shortening of calcium transient (less than 1 sec) within 20 day.
Hepatic-like cells on a chip were obtained by early endoderm commitment, definitive endoderm specification and hepatic cell-like maturation. Cells showed high expression of cytokeratin 18, CYP-3A and albumin, and indocyanine green uptake, glycogen storage capacity (75% of total cells) and albumin secretion. Compared to static conditions, we obtained higher hepatic markers expression, shortening of the time required for differentiation, 40% increase of albumin secretion.
In conclusion, we derived functional tissue-specific cells on a chip through a robust multi-stage microfluidic technology, which allows accurate control of cell soluble microenvironment. Differentiated cells integrated in the microfluidic channels can be directly used for temporal-defined drug screening and for micro-engineered disease modeling.

F-2093

CHEMICALLY DEFINED MEDIUM FOR MURINE TROPHOBLAST STEM CELLS

Caroline Kubaczka[1], Neha Sharma[1], Peter Kuckenberg[1], Astrid Becker[2], Marcos J. Araúzo-Bravo[4], Andreas Zimmer[2], Oliver Brüstle[3], Michael Peitz[3], Hubert Schorle[1]

[1]Developmental Pathology, Institute of Pathology, University of Bonn Medical School, Bonn, Germany, [A]Institute of Molecular Psychiatry, [B]Institute of Reconstructive Neurobiology, [2]Life and Brain Center, Bonn, Germany, [3]Max Planck Institute for Molecular Biomedicine, Münster, Germany

Trophoblast stem (TS) cells are the in vitro equivalents of the precursor cells of the placenta. In the mouse, TS cells can be derived either from polar trophectoderm (TE) of blastocyst outgrowths or from postimplantation extraembryonic ectoderm (ExE), originating from polar TE. Since their first successful derivation in 1998 TS cells are cultured in serum-rich medium in the presence of fibroblast growth factor 4 (FGF4), the cofactor heparin and fibroblast conditioned medium. Here, we tested a simple medium formulation for TS cell culture, previously used for human induced pluripotent stem (iPS) cell derivation and culture by Chen et al., 2011 in which all protein reagents for liquid media are chemically defined. After adapting the growth factor composition for the requirements of TS cells, the defined medium consists of only ten ingredients (TX-medium). TX supports growth and self-renewal of TS cells grown on matrigel-coated dishes over multiple (>10) passages. TS cells cultured under these conditions express key trophoblast markers, maintain their ability to differentiate into all derivatives of the trophectodermal lineage in vitro and give rise to haemorrhagic lesions in nude mice, indistinguishable from cells grown in standard conditions. Global gene expression profiling of cells cultured in both media conditions revealed, that 99.6 % of genes are

similarly expressed. Besides the culture of already established TS cell lines in defined media, new TS cell lines could be derived directly in defined medium conditions. The fact that TX media formulation no longer requires FCS and conditioned medium facilitates and standardizes the culture of the extraembryonic trophectoderm lineage in vitro.

iPS Cells

F-2101

GENETIC CORRECTION OF A LRRK2 MUTATION IN IPS CELLS LINKS PARKINSONIAN NEURODEGENERATION TO ERK-DEPENDENT CHANGES IN GENE EXPRESSION

Peter Reinhardt[1], Benjamin Schmid[2], Lena Burbulla[2], David C. Schöndorf[2], Lydia Wagner[1], Michael Glatza[1], Susanne Höing[1], Gunnar Hargus[1], Rejko Krüger[2], Tanja Kuhlmann[3], Thomas Gasser[2], Hans R. Schöler[1], Jared Sterneckert[1]

[1]Max-Planck Institute for Molecular Biomedicine, Muenster, Germany, [2]Hertie-Institute for Clinical Brain Research, Tübingen, Germany, [3]Institute of Neuropathology, University Hospital Muenster, Muenster, Germany

The *LRRK2* mutation G2019S is the most common genetic cause of Parkinson's disease (PD). To better understand the link between mutant LRRK2 and PD pathology, we derived induced pluripotent stem cells from PD patients harboring *LRRK2* G2019S and then specifically corrected the mutant *LRRK2* allele using zinc finger nucleases.
We demonstrate that gene correction resulted in phenotypic rescue in differentiated neurons, including stress induced apoptosis and decreased neurite outgrowth. Whole genome expression profiling uncovered gene expression changes associated with *LRRK2* G2019S, and knockdown experiments demonstrated that four of these genes (*CADPS2, CPNE8, MAP7* and *UHRF2*) contribute to dopaminergic neurodegeneration. Furthermore, *LRRK2* G2019S induced increased extracellular-signal-regulated kinase 1/2 (ERK) phosphorylation. We demonstrate that transcriptional dysregulation of three genes was dependent on ERK activity. We showed that PD-associated neurodegeneration and neurite outgrowth were ameliorated by pharmacological inhibition of ERK.
Finally, we developed this PD model into a system compatible with high-throughput screening (HTS) using neural precursor cells that are dependent only on small molecules for proliferation (smNPCs). This HTS-compatible model recapitulates PD - relevant phenotypes including the ERK-dependent disease mechanisms.

F-2102

GENERATION OF FUNCTIONAL NOCICEPTIVE NEURONS FROM BLOOD DONOR DERIVED IPS CELLS SUITABLE FOR MODELING PAIN AND SENSORY NEURON DISEASES

Anja Nitzsche, Anna Wilbrey, Alex Gutteridge, Rita Ferreira, Peter Stacey, Lishuang Cao, Gareth Young, Pierre-Phillipe Saintot, Alexandre Loucif, Edward Stevens, Paul Whiting, James Bilsland
Pfizer Neusentis, Cambridge, United Kingdom

Induced pluripotent stem cells (iPS cells) have become an important tool for disease modeling and are a potential source for cellular therapies. In this respect, human blood is an attractive cell source for non-invasive tissue collection of patients for disease modelling across multiple therapeutic areas including pain and neurosensory disorders. To date iPS generation from peripheral blood mononuclear cells (PBMCs) is highly inefficient with a number of drawbacks like time, yield, cell number and immunogenicity. Here, we report the successful iPS cell generation using PBMC derived erythroblasts in a simple way providing a sufficient amount of cells.
Culturing PBMCs in an erythroblast growth promoting cocktail followed by a fast Percoll density centrifugation provides a sufficient amount of cells in 10 days. In combination with a footprint free and xenofree reprogramming strategy using Sendai virus, we reproducibly harvested iPS colonies as fast as 17-21 days that express pluripotency markers comparable to human ES cells (hES) and exhibited a normal karyotype. Global mRNA expression using RNAseq showed a similar expression profile compared to H9 hES as well as iPS lines generated from fibroblasts.
Differentiation of iPS clones into sensory like neurons using established protocols (Chambers et al., 2012) confirmed expression of key sensory neuron markers (BRN3a, ISL1, PRPH) and pain-related ion channels (P2RX3, SCN9A,

SCN10A). The neurons generated are viable and display functional responses such as calcium flux in response to stimulation with alpha, beta-methylene ATP (P2X3 agonist) and KCl.
Electrophysiological analysis using whole cell patch-clamp technique demonstrated functional expression of the key sodium channels $Na_v1.8$ and $Na_v1.7$, which were sensitive to pharmacologic blockade using selective inhibitors. Inhibition of $Na_v1.7$ and $Na_v1.8$ altered action potential generation demonstrating the utility of these blood derived iPS derived neurons to support drug discovery in pain.

F-2103

GENERATION OF FUNCTIONAL PLATELETS FROM CANINE INDUCED PLURIPOTENT STEM CELLS

Toshiya Nishimura[1], Shingo Hatoya[1], Ryoji Kanegi[1], Kikuya Sugiura[1], Viskam Wijewaedana[1], Mitsuru Kuwamura[2], Miyuu Tanaka[2], Masahiro Takanashi[1], Noritoshi Kawate[1], Hiromichi Tamada[1], Hiroshi Imai[3], Toshio Inaba[1]
[A]Advanced Pathobiology, [B]Integrated Structural Biosciences, [1]Osaka Prefecture University, Izumisano, Japan, [2]Applied Biosciences, Kyoto University, Izumisano, Japan

Thrombocytopenia (TTP) is a common acquired hemostatic and platelet disorder in humans, and the disease is also found in dogs. Although blood transfusion is often used clinically as an effective therapy for TTP, blood contribution from donors are limited, and the life-span of platelets is very short outside the body. Other feasible methods should therefore be explored. In order to resolve this issue, we generated canine induced pluripotent stem cells (ciPSCs) from canine embryonic fibroblasts, and a novel protocol for creating functional platelets from ciPSCs. First, we generated ciPSCs by using lentiviral vectors containing four human factors (OCT4, SOX2, KLF4 and C-MYC). Our ciPSCs displayed similar characteristics to canine embryonic stem cells (cESCs) with dome shaped and a critical pluripotency marker, REX1, which is a marker specific to ESCs. Additionally, ciPSCs differentiated into cells derived from three germ layers via the formation of an embryoid body. Next, we differentiated the ciPSCs on OP9 cells which induce iPSCs to differentiate into hematopoietic progenitor cells with vascular endothelial growth factor. Inflated sac-like structures appeared in the culture medium on day 12-14 and these structures contained round cells like hematopoietic progenitor cells. On day 15, the cells were reseeded on fresh OP9 layers with several cytokines: human thrombopoietin, canine stem cell factor and heparin. After 24 h from reseeding, we found non-adherent cells expressed CD34 and megakaryocyte (MK) -like cells expressed CD41/61 (GP IIb/IIIa). Also, we found platelet-like particles expressing CD41/61, a specific marker for platelets and MKs. As expected, therefore, megakaryocytic cells derived from ciPSCs released platelets into the supernatant of culture. Electron microscopic examination revealed that the particles were oval in shape, with ultrastructures like open canalicular system (OCS) and α-granules. These features were specific to platelets and were crucially similar to the structure of canine peripheral platelets. To examine the function of the particles, we added platelet-activating agonists, thrombin and adenosine diphosphate (ADP) to activate the αIIbβIII receptor required for platelet aggregation (inside-out signaling). The flow cytometric analysis indicated that platelets from ciPSCs could bind to FITC-fibrinogen in the presence of ADP or thrombin as is the case of functional platelets. Taking these results together, it is indicated that we successfully generated hematopoietic progenitor cells and MKs from ciPSCs and that generated MKs finally differentiated into platelet in our culture system. These results indicate that the platelets generated from ciPSCs have morphological and functional properties similar to peripheral platelets, and could be leading useful model and a new therapy for TTP.

F-2104

USING NEURONS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS TO EVALUATE GAMMA-SECRETASE MODULATORS

Shauna Yuan, Qing Liu, Shannon Waltz, Steve Wagner
University of California, San Diego, La Jolla, CA, United States

Human induced pluripotent stem cells (iPSC) hold the potential to serve as a disease-in-a-dish model for designing drugs specifically for individual patients. However, this revolutionary potential has yet to be fully demonstrated

either in preclinical drug screening/testing or in the safety/toxicity evaluation of drug candidates. Application of this technology could be particularly rewarding in drug discovery/development for Alzheimer's Disease (AD), which is a neurodegenerative disorder causing dementia late in life with limited treatment options. The current systems commonly used for AD drug screening/testing and for lead compound identification and optimization are based on either peripheral cell-based assay systems requiring overexpression of the mutant Familial Alzheimer's Disease (FAD) genes, such as Amyloid precursor protein (APP) and/or Presenilin 1 and 2 (PS1, PS2), or non-human cell-based systems (e.g. murine neuroblastoma cell lines), which renders it difficult to interpret how the efficacies attained in these cell-based assay systems pertain to naïve human neuronal cells. Modulation of the γ-secretase complex via γ-secretase modulators (GSMs) is currently being investigated for the treatment of AD. These GSMs work by selectively lowering the levels of the most amyloidogenic Aβ 42 peptide and reducing the formation of amyloid plaques, one of the hallmarks of AD pathology and the basis of the amyloid cascade hypothesis. GSMs represent a promising new class of drugs, which modulate without inhibiting γ-secretase and are Notch-sparing. The PS1 mutation iPSC lines are an ideal supplement for this type of preclinical evaluation, because the PS1 mutations are known to cause elevated Aβ42/40 ratios at the endogenous level of expression, and in neurons, which are the main cell type at risk in AD. We tested two different GSM compounds in multiple cell types including primary human fibroblasts, neural stem cells (NSC) derived from iPSC and neurons derived from iPSC from normal control subjects and PS1 mutation carriers. When we measured the IC50 in fibroblasts and neurons, we found that the GSMs were highly effective in the neurons, with IC50 in the nM range; however, they were not as effective in the fibroblasts. We then observed that while GSM treatment of the fibroblasts led to reduction of the Aβ42 and Aβ40 and elevation of the Aβ38 levels, consistent with previous observation in over-expressing APP cell lines and APP transgenic mice, GSM treatment of NSC and sorted neurons led to reduction of Aβ42 and Aβ40 but also reduction of Aβ38. Measurement of total Aβ in NSC and neurons revealed that total Aβ was unchanged; suggesting that GSM does not act as an inhibitor. These results suggest that APP processing by γ-secretase could be different in NSC and neurons compared to non-neuronal cells or APP overexpressing cells. This is the first demonstration of effectiveness of GSM in human neurons with endogenous copies of mutant PS1. This also demonstrates that it would be possible to use this system for drug selection and pre-clinical drug evaluation and optimization.

F-2105

AN EFFICIENT NON-VIRAL METHOD TO GENERATE INTEGRATION-FREE HUMAN IPS CELLS FROM CORD BLOOD AND PERIPHERAL BLOOD CELLS

Keisuke Okita, Tatsuya Yamakawa, Yasuko Matsumura, Yoshiko Sato, Naoki Amano, Akira Watanabe, Shinya Yamanaka
Dept. of Reprogramming Science, CiRA, Kyoto Univ, Kyoto, Japan

The generation of induced pluripotent stem cells (iPSCs) provides the opportunity to use patient-specific somatic cells, which are a valuable source for disease modeling and drug discovery. Human iPSCs can be generated from a wide spectrum of somatic cells, including fibroblasts, keratinocytes, and mesenchymal stem cells in adipose tissue. Among them, cord blood cells and peripheral blood mononuclear cells are attractive sources to use for the generation of iPSCs because of the low invasiveness of their collection. We have recently reported the efficient generation of human iPSCs from adult fibroblasts using a combination of plasmids encoding OCT3/4, SOX2, KLF4, L-MYC, LIN28 and shRNA for TP53. These factors are encoded in widely used vectors which have two components of the Epstein-Barr virus, OriP and EBNA1. The EBNA1 sequence encodes a protein and expresses it from its viral promoter after transduction into human somatic cells. The EBNA1 protein in turn recognizes the OriP sequence and induces plasmid amplification coincident with DNA amplification of the host cell. This system enables relatively high and long term expression of the reprogramming factors. In this conference, we will report a modified protocol enabling efficient iPSC induction from CD34+ cord blood cells and from peripheral blood using these plasmid vectors. The aforementioned plasmid mixture could induce iPSCs, however, the efficiency was low. The addition of EBNA1 by an extra plasmid greatly increased the efficiency of iPSC induction, especially when the induction was performed from αβT cells. The iPSCs had genomic rearrangement at the TRB and TRD loci, indicating their T cell origin. We also established iPSCs which did not show any evidence of genomic rearrangement in the TRB, TRD, and IGH loci by

changing the culture medium. Most of these iPSCs were integration-free, karyotypically normal, and effectively differentiated into various cell types in vivo. To the best of our knowledge, this method was the most efficient simple method to generate iPSCs from peripheral blood by a non-viral vector. This represents a reliable method to generate patient-specific iPSCs, and would be applicable to the generation of clinical grade iPSCs in the future.

F-2106

COMPARISON OF ISOGENIC HUMAN ESC AND IPS CELL LINES.

Maria V. Shutova[1], Anastasia V. Surdina[1], Alexander Panchin[2], Maria A. Lagarkova[1], Sergey L. Kiselev[1]
[1]epigenetics, VIGG RAS, Moscow, Russian Federation, [2]epigenetics, Institute for Information Transmission Problems RAS, Moscow, Russian Federation

The question about the identity of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSC) arose after the first experiment of direct reprogramming of fibroblasts to pluripotent state, and is being actively discussed until now. In order to minimize potential differences that could be introduced in the reprogrammed cells by somatic cells individual genetic background we established an inducible reprogramming system enabling reversion to the pluripotent state an hESCs-derived differentiated cells of an ectodermal or mesodermal origin. The resulted hiPS cells were similar to the hESCs in their molecular and functional properties. To reveal epigenetic differences stipulated by reprogramming we performed DNA methylation analysis of 2 parental hESC lines, 3 hESC-derived differentiated lines, and 6 hiPSC lines with the Infinium HumanMethylation450 BeadChip. Overall our data indicate high level of identity of parental hESC and hiPSC lines. However, differences in methylation pattern of 8 genes were detected. Most of them were metabolic genes, and their methylation level did not influenced their expression, according to Illumina HT12 transcription profiling. However, only one gene, MEG3, showed higher methylation level and lower expression in all hiPS cells. At the same time, we observed individual differences between pairs of hiPSC lines from different origins. That can be explained in part by somatic memory, and in part - as a fingerprints of stochastic events during reprogramming. Nevertheless, we demonstrated that only MEG3 gene hypomethylation could be considered as a uniform trace of reprogramming process.

F-2107

GRAINY HEAD LIKE FACTOR REGULATES SOMATIC CELL REPROGRAMMING

Sumitha P. Bharathan, Dhava Priya, Kannan V. Manian, Syed Mohammed Musheer Aalam, Alok Srivastava, Shaji R. Velayudhan
Centre for Stem Cell Research, Vellore, India

Grainy head like factor-2 (*GRHL2*) is one of the major transcription factors involved in epithelial specific transcriptional regulation of genes. Its overexpression causes induction of epithelial gene expression in disseminated cancer cells and its downregulation causes epithelial mesenchymal transition (EMT). It has also been identified as a transcription regulator of hTERT gene and enhances replicative potential of human keratinocytes and inhibits its differentiation. GRHL2 is a component of OCT4 interactome, which determines pluripotency of embryonic stem cells. We measured *GRHL2* transcript levels in dermal fibroblasts before and after transduction with *OCT4, SOX2, KLF4* and c*MYC* (OSKM) and induced pluripotent stem cells (iPSCs) and found that while *GRHL2* is not expressed in fibroblasts, it is expressed in very high levels in iPSCs and 6 days after transduction of fibroblasts the level was 50% of that in iPSCs, which suggests that activation of GRHL2 is a major event in initial stages of reprogramming. Due to its properties of inhibition of EMT, activation of epithelial specific genes, interaction with pluripotency regulators and activation during reprogramming process, we hypothesized that overexpression of *GRHL2* can accelerate and increase efficiency of somatic cell reprogramming. We amplified human *GRHL2* cDNA from human embryonic stem cells and cloned into a retroviral vector and reprogramming was carried out by overexpression of *GRHL2* along with *OCT4, SOX2, KLF4* and c*MYC* (OSKM+G) in normal adult dermal fibroblasts. The cells were also transduced with red fluorescent protein (*RFP*) to monitor retroviral transgene silencing, which occurs in fully pluripotent iPSC colonies. The efficiency of reprogramming was estimated on 15 days after transduction on the basis of TRA-I-60 expression,

retroviral transgene silencing and morphology and size of the colonies. We observed a striking difference in size and number of hiPSC colonies formed in OSKM and OSKM+G experiments. Unexpectedly, OSKM+G experiment showed approximately 50% decrease in the number of Tra160+RFP- colonies (59 vs 117) and about 24% decrease in the size of colonies formed (avg surface area of the colonies: $0.13mm^2$ vs $0.17mm^2$), compared with OSKM. Reprogramming experiment by knocking down GRHL2 expression by using shRNAs (OSKM+shG) showed a reduction in efficiency by 31% (80 vs 117 colonies). On day15, the reprogramming efficiencies were found to be 0.14%, 0.07% and 0.096% with OSKMR, OSKM+G and OSKM+shG, respectively. On day 18, when most of the colonies achieved defined morphology of iPSCs, the efficiencies estimated based on NANOG expression were 0.25%, 0.15% and 0.1%. This indicates that although GRHL2 is expressed on 6^{th} day in almost all OSKM transduced cells and it has most of the properties of an activator of reprogramming, its overexpression reduces the reprogramming efficiency significantly. The basis of reduced reprogramming efficiency in OSKM+G may be due to the fact that although GRHL2 may favour MET in the initial stages of reprogramming, its constitutive ectopic expression inhibits the cells from going to the next stage of reprogramming. The reduced efficiency observed with GRHL2 knockdown shows that it is indeed an activator of reprogramming. Our results suggest that GRHL2 is one of the stage specific regulators of reprogramming. Inducible knocking down and overexpression system will help us to understand the roles of these regulators in different stages of reprogramming.

F-2108

VARIEGATED EXPRESSION OF IMPRINTED GENES IN MOUSE INDUCED PLURIPOTENT STEM CELLS

Syed Mohammed Musheer Aalam, Kannan V. Manian, Sumitha P. Bharathan, Alok Srivastava, Shaji R. Velayudhan
Centre for Stem Cell Research, Vellore, India

Most of the generated mouse induced pluripotent stem cells (iPSC) clones show embryonic stem cell (ESC) like features of expression of pluripotency genes and the ability for *in vitro* and *in vivo* differentiation. However, many clones do not support the full term development of tetraploid-complemented mice. Recent studies have shown that transcripts present in the imprinted *Dlk1-Dio3* gene cluster on chromosome 12qF1 are aberrantly silenced in most of the iPSC clones which causes poor contribution to chimeras. Decreased expression of these imprinted genes has been attributed variously to sub optimal reprogramming factor stoichiometry and loss of active chromatin marks or hypermethylation of this locus. Addition of Vitamin C and downregulation of *Dnmt3a/3b* have been shown to help in maintaining the normal imprinting of the genes in this locus. It has also been proposed that Klf4 and Oct4 regulate the expression of these imprinted genes and the overexpression of Klf4 and Oct4 causes their normal expression. We estimated the expression levels of *Gtl2, Rian* and *Mirg* in the *Dlk1-Dio3* imprinted cluster in various mouse iPSC clones in our laboratory to test whether it can be used as an effective and quick method to assess the pluripotency of mouse iPSC clones generated using retroviral transduction of four factors, Oct4, Sox2, Klf4 and c-Myc (OSKM) or three factors, Oct4, Sox2 and Klf4 (OSK). We included *mRFP1* for transduction to monitor retroviral transgene silencing, which occurs in pluripotent clones. The transgene silenced (RFP-) clones (n=7) had activated expression of endogenous pluripotency genes *Oct4, Sox2*, *Klf4* and *Nanog* to the levels similar to those in R1ESCs while RFP+ clones (n=8) maintained high expression levels of all the transgenes and had no induction of endogenous pluripotency genes except for *Oct*4. The RFP- Nanog+ clones showed variegated expression of *Rian, Gtl2, Mirg*; 2 out of 4 OSKM clones had high levels of *Gtl2* and *Mirg* expression but lacked the expression of *Rian* and the remaining two clones lacked the expression of all three imprinted genes. Out of the 3 OSK clones, 2 clones expressed optimal levels of *Rian* and *Mirg*, but they lacked expression of *Gtl2* and the other one expressed only *Rian*. In 7 out of 8 RFP+ clones, the imprinted genes were silenced and in one of them only *Gtl*2 was expressed. We could not find any correlation between expression of *Klf4* and *Dlk1-Dio3* cluster genes. We measured the expression levels of *Hdac*1 to 11 and the results showed that *Hdac*7 is repressed in R1ES cells and RFP- iPSCs but is highly expressed in RFP+ iPSCs and mouse embryonic fibroblasts (MEFs) and in contrast, *Hdac*1 was found to be repressed in MEFs and activated at comparable levels in R1ESCs and in both RFP- and RFP+ iPSC colonies. However, there was no correlation between the expression levels of *Hdac*1 or *Hdac*7 and the imprinted genes. These results suggest that downregulation of *Hdac*7 is important for reprogramming, but not sufficient to activate the expression of imprinted genes in iPSC clones. VPA causes increase in acetylation of imprinted genes, but not by directly modulat-

ing the levels *of Hdac1* and *Hdac*7. We conclude that there is heterogeneity in the expression of *Dlk1-Dio3 cluster* genes and they are not transcriptionally co-regulated and different epigenetic mechanisms may control the individual expression of these genes. A thorough understanding of the factors responsible for the activation of these genes will help us tailor strategies to obtain fully pluripotent mouse iPSC clones.

F-2111

NOVEL STAGES OF REPROGRAMMING REVEALED BY STUDIES OF X CHROMOSOME ACTIVATION

Vincent Pasque[1], Jason Tchieu[1], Dana Case[2], Anu Sadhu Dimashkie[2], Sanjeet Patel[2], Bernadett Papp[2], Takashi Sado[3], Takashi Tada[4], Kathrin Plath[2]

[1]University of California Los Angeles, Los Angeles, CA, United States, [B]Biological Chemistry, [1]University of California Los Angeles, Los Angeles, CA, United States, [2]Kyushu University, Fukuoka, Japan, [3]Kyoto University, Kyoto, Japan

The epigenetic state of somatic cells can be entirely reversed to a pluripotent state, however this process is poorly understood. Somatic cell reprogramming to mouse induced pluripotent stem cells (iPSCs) leads to the complete reactivation of the developmentally inactivated X chromosome (Xi), but underlying mechanisms have remained unclear. Using a detailed time-course analysis of Xi chromatin states and pluripotency factor activation at the single-nucleus level, we visualized Xi-chromatin dynamics during reprogramming and unravelled multiple sequential epigenetic steps upstream and downstream of the expression of the key pluripotency factor Nanog. Transitions between these steps occur in cells progressing towards pluripotency as they can be recapitulated with isolated reprogramming intermediates. Remarkably, within early intermediates, but not within late intermediates, changes in chromatin structure on the inactive X chromosome proceed in an inverse order of developmentally regulated X-inactivation. The divergence late in reprogramming is best explained by the fact that DNA demethylation of genes on the inactive X only takes place very late, after demethylation of the Nanog promoter, and coincides with gain of 5-hydroxymethylcytosine. Consequently, both DNA methylation and Xist RNA coating mechanistically maintain silencing of the Xi until late in reprogramming, making the reactivation of the X one of the latest events of the reprogramming process. Nanog is required for the initiation of a cascade of events leading to X reactivation and the establishment of complete naïve pluripotency. Together, these findings reveal an unprecedented molecular detail of the reprogramming process, and establish that transitioning through a multitude of hierarchical, phased epigenetic changes is a fundamental feature of X chromosome reactivation and of somatic cell nuclear reprogramming.

F-2112

MATURATION IS A MAJOR LIMITED STEP OF REPROGRAMMING TOWARD PLURIPOTENT STATE, BUT NOT INITIATION.

Koji Tanabe, Michiko Nakamura, Megumi Narita, Kazutoshi Takahashi, Shinya Yamanaka
CiRA, Kyoto university, Kyoto, Japan

Pluripotency can be induced in somatic cells by forced expression of OCT4, SOX2, KLF4, and MYC. However, it is generally seems that factor-mediated direct reprogramming is inefficient, slow and stochastic event. Here, we found that at the early stage, not only very tiny population of transduced cell was initiated reprogramming, but also large population (~20%) of transduced cells acquire a partially reprogrammed state in which cells was positive for TRA-1-60(+). However, just small population of these partial reprogrammed cells (~2%) acquired further pluripotent state and formed induced pluripotent stem cell (iPSC) colonies. Then this gap raises an important question, "why only a small portion of partial reprogrammed cells can become iPSCs?" We investigated the reprogramming intermediate states using TRA-1-60(+) cells in gene expression circumstantially. We identified two mechanisms for the failure

in maturation of the de novo reprogrammed cells: Many of TRA-1-60 (+) cells turned back to be negative or undergo apoptosis during the subsequent culture. Among factors that had been reported to promote direct reprogramming, Lin28 and Glis1 significantly inhibited the reversion of reprogramming, but not NANOG, Cyclin D1 or p53 shRNA. In contrast, Glis1 and p53 shRNA counteracted apoptosis. These data demonstrated that maturation, but not initiation is bottleneck during direct reprogramming toward pluripotency and that each pro-reprogramming factor has a different mode of action.

F-2113

TRANSIENT C/EBPA EXPRESSION IN PRE-B CELLS ELIMINATES STOCHASTIC PHASE OF IPS CELL REPROGRAMMING

Bruno Di Stefano[1], Chris van Oevelen[1], Eric Kallin[1], Samuel Collombet[2], Thomas Graf[1]

[1]Gene Regulation, Stem Cells and Cancer Program, Center for Genomic Regulation (CRG); Universidad Pompeu Fabra; and Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain, [2]UMR ENS-CNRS 8197-INSERM 1024, Institute of Biology of the Ecole Normale Supérieure (IBENS), Paris, France

Reprogramming of somatic cells into iPS cells by Oct4/Sox2/Klf4/Myc (OSKM) occurs at very low frequencies and requires approximately 2 weeks for the full expression of the pluripotency programme. Therefore it has been postulated that cell reprogramming consists of a stochastic and a hierarchic phase. C/EBPalpha is a transcription factor that very efficiently and rapidly transdifferentiates B cells into macrophages. We have now discovered that transient activation (18 hours) of C/EBPa in pre-B cells induces a 100-fold increase in OSKM-induced iPS cell colonies positive for Nanog expression. In addition, the C/EBPa pulse accelerates the process by 4 to 6 days as evidenced by the induction of OSKM transgene independence, retroviral silencing and X chromosome reactivation. Transcriptome analyses revealed the rapid activation (within 2 days) of endogenous Oct4 and of mesenchymal epithelial transition genes, with >80% of the cells expressing E-cadherin after 4 days. Nanog, Sox2, Esrrb, Sall4, Tet1 and other pluripotency genes become expressed 2 to 4 days later, reaching levels comparable to ES cells within approximately one week. Surprisingly, the activation of pluripotency genes is accompanied by the upregulation of many myeloid genes and OSKM induces in control pre-B cells a transient B cell to macrophage switch. Knocking down C/EBPa partially inhibits iPS reprogramming, consistent with a role of the factor in the process. Finally, the C/EBPa pulse induces full accessibility of Pou5f1 (Oct4) as well as other pluripotency genes to Oct4 DNA binding within 2 days after OSKM expression. Together, our findings support the notion that transient exposure of pre-B cells to C/EBPa largely eliminates the stochastic phase of iPS reprogramming.

F-2114

TAKING SNAP SHOTS DURING IPSC PRODUCTION BY SEVDP VECTORS

Ken Nishimura[1], Tetsuo Kato[1], Lalhaba Oinam[1], Takahito Narita[1], Manami Ohtaka[2], Aya Fukuda[1], Mahito Nakanishi[2], Koji Hisatake[1]

[1]Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan, [2]Center for Stem Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan

For the efficient production of well-reprogrammed induced pluipotent stem cells (iPSCs), it needs the understanding of its detail mechanism. However, during their production, forced expression of several transcription factors induces continuous and very complex change of gene expression in the cells. The expression balance of the factors affects quality of iPSCs, but it is difficult to control the balance in the conventional iPSC production methods because the factors are introduced separately from each vector. So, the conventional methods are not suitable for the analyses of such a complex change during iPSC production.
We have developed unique gene transfer system based on persistent mutant strain of Sendai virus. The vectors, named SeVdp vectors, remain persistently in the cytoplasm without integrating into the host genome and also en-

able transfer and expression of multiples genes with fixed balance from single vector genome (Nishimura, K. et al. 2011). These properties make it exceptionally suitable for production of iPSCs. Indeed, an SeVdp vector harboring four reprogramming factors (Oct4, Sox2, Klf4 and c-myc) can reprogram mouse and human somatic cells very efficiently.
In this presentation, we report analyses of iPSCs induced by different SeVdp vectors which express the reprogramming factors with different expression balance. We prepared some vectors expressing the four factors with different expression balance and used them for cell reprogramming. Investigating ES cell-specific marker gene expression in the induced colonies by each vector showed that the difference of the expression balance caused different expression pattern of the marker genes in the iPSCs, although all vector expressed same four factors. Among the four factors, strength of Klf4 expression particularly affects iPSC quality, and the vector with low Klf4 expression only induced partially reprogrammed cells. Moreover, up-regulation of Klf4 expression in the partially reprogrammed cells proceeded the cell reprogramming and enabled us to establish well-reprogrammed iPSCs from them. These results indicates that the change of Klf4 expression can induce several types of cells stopped at different stage of cell reprogramming and these partially reprogrammed cells seemed to be used as 'snap shots' of iPSC production.

F-2115

USING HUMAN IPS CELLS TO STUDY MOTOR NEURON DEGENERATION IN SPINAL MUSCULAR ATROPHY

Yong-Chao Ma, Nimrod Miller
Northwestern University Feinberg School of Medicine, Chicago, IL, United States

Spinal Muscular Atrophy (SMA) is an autosomal recessive genetic disorder characterized by the loss of spinal motor neurons and by proximal muscle atrophy, leading to paralysis, decreased respiratory function, and ultimately death. As the leading genetic cause of infant mortality, SMA affects one in every 6000 live births. SMA is caused by mutations in the survival motor neuron (smn) gene in humans. However, the underlying pathogenic mechanism of SMA is poorly understood, and there is no efficient treatment for SMA.
In this study using induced pluripotent stem (iPS) cells from SMA patients' fibroblasts as a unique model, we found a novel pathogenic mechanism and treatment strategy for SMA. In human SMA iPS cell-derived motor neurons, we found that axon transport is significantly slowed down. This is caused by increased activity of cyclin-dependent kinase 5 (Cdk5) and its phosphorylation of histone deacetylase 5 (HDAC5) on a novel phosphorylation site, Serine 279 (S279), within the nuclear localization sequence. This phosphorylation event leads to increased cytoplasmic localization of HDAC5, decreased acetylation of microtubule tubulin, and compromised motor axon transport. AAV9-mediated expression of Smn gene in human SMA iPS cells rescues axon transport and motor neuron degeneration phenotypes, suggesting a new therapeutic strategy. Importantly, these pathogenic mechanisms are conserved in SMA mouse models. Together, our studies demonstrate human iPS cells as a unique model to study molecular mechanisms regulating motor neuron axon transport and motor neuron degeneration. These findings not only have implications for the development of new therapeutics for SMA, but also for other neurodegenerative disorders.

F-2116

SMALL MOLECULES PROMOTE IPS CELL GENERATION VIA INDUCTION OF THE MIR-302/367 CLUSTER

Wen-shu Wu, Zhonghui Zhang
Jordan Family Center for Blood & Marrow Transplantation and Cellular Therapies, Children's Hospital Oakland Research Institute (CHORI), Oakland, CA, United States

MicroRNAs and small molecules (for example butyrate) can greatly enhance the efficiency of iPS cell generation. It have been reported that sodium butyrate (NaB) enhances histone H3 acetylation and promotes DNA demethylation and the expression of endogenous pluripotency-associated genes during reprogramming. We previously showed that a small cocktail (NaB, PD0325901, and SB431542) selectively promote generation of fully reprogrammed human iPS cells. However, the molecular mechanisms of how small molecules promote cellular repro-

gramming remain largely unknown. MicroRNAs are 18-24 nucleotide single-stranded RNAs and play important roles in regulating the self-renewal and differentiation of human embryonic stem cell (hES) cells, as well as regulating cellular reprogramming. So far, roles of microRNAs in the regulation of small molecule-mediated reprogramming have not been explored. In our current study, we investigated how small molecules promote reprogramming through the regulation of the miR-302/367 cluster, which is highly expressed in hES cells and promote iPS cell generation when overexpressed. Here, we showed that a small molecule cocktail (NaB, PD0325901, and SB431542) upregulate expression of the miR-302/367 cluster by increasing RNA stability and transcription level. Interestingly, Oct4 is required for upregulation of the miR-302/367 cluster by the small molecule cocktail. Further, we showed that NaB enhances Oct4 transcriptional activity in the miR-302/367 promoter. By luciferase reporter assay using different mutant Oct4, we revealed that NaB does not affect Oct4 binding activity, but decrease its transactivity. Our co-immunoprecipitation assay further suggests that Oct4 associates with the histone deacetylases (HDAC) complex and NaB diminishes the association between Oct4 and the HDAC complex. To determine if the miR-302/367 is required for small molecules to promote cellular reprogramming, we reduced activity of the miR-302/367 cluster by using lentivirus-mediated stable microRNA inhibition and a specific transcription activator-like effector (TALE)-transcription repressor. Our data showed that attenuation of the miR-302/367 cluster impairs ability of small molecules in promoting cellular reprogramming. Last, we deleted the miR-302/367 cluster in primary human fibroblasts by using two pairs of TALE nucleases designed in our laboratory. We demonstrated that knockout of the miR-302/367 cluster blocks iPS cell generation in conditions with the small molecule cocktail. Collectively, Our data suggest that enhancement of reprogramming by the small molecule cocktail is mediated at least partly by its effects on expression of the miR-302/367 cluster. Our findings provide new insights into molecular mechanisms of how small molecules promote reprogramming process, thus may pave the way for generation of iPS cells using only small molecules.

F-2117

ALPHA-1 ANTITRYPSIN DEFICIENT IPSCS REVEAL DISEASE-SPECIFIC GLOBAL TRANSCRIPTOMES AND THERAPEUTIC TARGETS

Andrew A. Wilson[1], Lei Ying[2], JC Jean[1], Steven Shen[3], Avrum Spira[3], Paul Gadue[2], Darrell N. Kotton[1]

[1]Center for Regenerative Medicine (CReM), Boston University School of Medicine and Boston Medical Center, Boston, MA, United States, [2]The Children's Hospital of Philadelphia, Philadelphia, PA, United States, [3]Internal Medicine, Boston University School of Medicine, Boston, MA, United States

Objectives: The derivation of disease-specific induced pluripotent stem cells (iPSC) provides unprecedented opportunities to understand the pathogenesis of inherited disease in each patient's own genetic background. We sought to derive transgene-free iPSC from individuals with inherited alpha-1 antitrypsin deficiency (AATD) in order to develop disease models and therapeutic screens for AATD in iPSC-derived hepatocytes. We recruited 3 normal volunteers as well as 3 individuals with the most common genotype responsible for AATD, namely homozygous mutant Z alleles encoding the AAT protease inhibitor, hereafter PiZZ.

Methods/Results: We sought to test the hypothesis that a single iPSC clone from each donor could be used to detect disease-specific differences between the normal cohort and the PiZZ cohort, and we anticipated that a difference would emerge only at a developmental stage in which the mutant AAT gene is expressed, namely in differentiated hepatocytes. Employing single iPSC clones from each normal or PiZZ individual as well as normal ESC control clones (n=3 per group), we monitored all 9 cell lines across three time points of hepatic directed differentiation, representing 3 developmental stages: undifferentiated (T0), definitive endoderm (T5), and early hepatocyte (T24). No significant differences in differentiation efficiency were observed between PiZZ and normal lines, as measured by flow cytometry quantitation of protein expression of pluripotency (SSEA3, TRA-1-60), definitive endoderm (CKIT/CXCR4) and hepatic (AAT/FOXA1) markers at each developmental stage. At hepatic stage (T24) we found intracellular accumulation of mutant, misfolded AAT protein in all 3 of our PiZZ iPSC-derived hepatocytes. We prepared RNA extracts from all 9 lines at T0, T5, and T24, and we used Affymetrix microarrays to compare gene expression across these 27 samples by 2 way ANOVA, screening for differential gene expression that might distinguish cell types or

developmental stages. We found that ESC, iPSC, and PiZZ iPSC did not differ in either the undifferentiated or endodermal stages, but PiZZ iPSC exhibited a distinct gene expression profile only upon reaching hepatocyte stage (T24). This signature of approximately 80 differentially expressed genes we have named the PiZZ hepatic disease-specific signature, which reveals evidence of pathways of apoptosis and oxidant stress activated in the diseased hepatocytes. Importantly there were no transcriptomic differences between ESC and normal control iPSC at any developmental stage. Next we tested the efficacy of experimental therapeutics to ameliorate disease severity in our iPSC AATD model. As has been predicted by mouse studies and heterologous cell models, we established that the autophagy-augmenting drug, carbamazepine displayed efficacy in reducing the burden of intracellular accumulation of AAT protein.
Conclusion: PiZZ disease-specific iPSC in the undifferentiated state express a global transcriptome essentially indistinguishable from control normal iPSC or ESC, but display a disease-specific global transcriptome signature after differentiation into hepatic lineage cells. These iPSCs allow disease modeling and drug testing of novel therapeutics for the treatment of AATD.

F-2118

MODULATION OF NUCLEAR REPROGRAMMING FOR IPSC GENERATION WITH ADDITIONAL FACTORS

Abbas Beh-Pajooh[1], Axel Schambach[2], Tobias Cantz[3]

[1]Stem Cell Biology, Cluster of Excellence REBIRTH, Hannover Medical School, Germany, Hannover, Germany, [2]Institute of Experimental Hematology, Hannover Medical School, Germany, Hannover, Germany, [3]Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, Münster, Germany, Hannover, Germany

Introduction

Generating induced pluripotent stem cells (iPSCs) is a useful method to avoid destroying blastocysts to obtain their inner cell mass as an unlimited resource for pluripotent stem cells. However, the reprogramming of somatic cells into iPSCs takes significantly more time than the nuclear transfer-mediated reprogramming of somatic cells. Knock-down of Cdx2 during nuclear transfer not only avoided the generation of totipotent embryos but more importantly dramatically increased the efficiency of pluripotent stem cell generation.

We investigated the knock-down of Cdx2 during nuclear reprogramming for iPSC generation and speculated that Cdx2 knock-down might further promote the establishment of pluripotency-associated transcriptional networks.

Methods & Results

We were able to demonstrate Cdx2 expression by qRT-PCR during reprogramming of mouse embryonic fibroblasts, starting from day 9 in an ascending trend. When we applied a lentiviral construct carrying a miR-30-styled shRNA-construct for the knockdown of Cdx2 during iPSC generation, we could suppress Cdx2 expression until day 12. However, after full establishment of iPSC colonies the SFFV-promoter driven knockdown construct was silenced as well as the reprogramming vectors. With respect to morphology, the number, and the time of iPSC colonies appearance we could determine major effects of the Cdx2 knockdown.

Conclusion

In our experiment we demonstrated that Cdx2 is moderately expressed in the late phase of iPSC reprogramming. However, applying an efficient knockdown strategy during the crucial days of reprogramming, we could not demonstrate an enhancing effect for iPSC generation.

F-2121

A DISEASE MODEL OF FAMILIAL HYPERTROPHIC CARDIOMYOPATHY USING PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

Shunsuke Funakoshi[1], Tadashi Takaki[2], Kenji Miki[2], Misato Nishikawa[2], Akiko Oishi[2], Ikue Takei[2], Takeru Makiyama[1], Takeshi Kimura[1], Shinya Yamanaka[2], Yoshinori Yoshida[2]

[1]Kyoto Univ, Kyoto, Japan, [2]Center for iPS Cell Research and Application, Kyoto, Japan

Hypertrophic Cardiomyopathy(HCM) is a popular inherited disease which leads to heart failure, arrhythmia and sudden cardiac death. Although several genetic mutations causing HCM have been identified, much phenotypic heterogeneity is evident between and within families carrying the same mutations. This implies that these mutations of cardiac sarcomeric proteins are not the sole determinant of HCM phenotype and prognosis. On the other hand, research about unknown mechanism of these inherited diseases using induced pluripotent stem cells(iPSCs)-derived disease model have been actively performed recently. However, in the field of heart disease model using iPSCs, it has been very difficult to recapitulate disease phenotype clearly due to the immaturity of iPSCs-derieved cardiomyocytes (iPSC-CMs) as compared to adult CMs. To overcome this problem and clarify the mechanisms of this HCM phenotypic heterogeneity, we generated patient-specific iPSC-CMs from members in families carrying a HCM causing missense mutation (Glu927Lys) in MYH7 gene and have analyzed the phenotype of these iPSC-CMs with and without any stimuli. All HCM-iPSC lines had normal morphological characteristics and expressed pluripotency-related genes comparable to ES cells and normal iPSCs. There were no significant differences between established iPSC lines in terms of the differentiation propensity into cardiomyocytes. In comparing the phenotype of iPSC-CMs without any stimuli, the ratio of disorganized sarcomeric CMs were significantly higher in case of HCM patients than in the case of healthy persons (36.3% versus 17.2%; $p<0.05$). However, HCM patients-derived iPSC-CMs did not show the apparent hypertrophic phenotype (cell size;2365um2 versus 2360um2, $p=0.96$). These results suggested that these iPSC-CMs could recapitulate a part of the HCM disease phenotype, not all the disease phenotype in vitro culture without any stimuli as is expected. Therefore, we now give several stimuli to iPSC-CMs, including chemical and mechanical modification. We want to introduce our methods in which we are trying to clarify the hypertrophic phenotype in the case of HCM patient iPSC-CMs.

When we can recapitulate disease phenotype clearly by stimulating cultured cells, this HCM disease model would be useful to research disease mechanism and identify new therapies.

F-2122

MODELING FRAGILE X SYNDROME WITH INDUCED PLURIPOTENT STEM CELLS TO DEFINE NOVEL THERAPEUTIC TARGETS.

Michael J. Boland[1], Kristopher L. Nazor[1], Ryder Paredes[2], Ha T. Tran[1], Jeanne F. Loring[1]
[1]The Scripps Research Institute, La Jolla, CA, United States, [2]California State University Channel Islands, Camarillo, CA, United States

Fragile X Syndrome (FXS; OMIM #300624) is the leading cause of inherited mental retardation in boys and contributes to a significant percentage of autism and autism spectrum disorders. It is attributed to expansion of a CGG trinucleotide repeat in the 5'-UTR of the FMR1 gene, leading to epigenetic silencing and loss of expression of the fragile X mental retardation protein (FMRP), which results in a range of cognitive and behavioral deficits. At the molecular level, FMRP is an mRNA binding protein thought to regulate protein synthesis in neuronal dendrites and synapses in the cortex, hippocampus and cerebellum. The neurons of FXS patients possess a higher density of immature dendritic spines, altered synaptic plasticity and aberrant electrophysiological properties. These findings indicate FMRP influences dendritic maturation, synaptic plasticity and neuronal function. Most studies and potential therapies have focused on the neuronal defects of FXS; however, recent studies have observed an altered neural differentiation potential of FXS-neural precursor cells (NPCs), in which gliogenesis is promoted relative to neurogenesis in the absence of FMRP. This suggests that the underlying molecular defects of FXS are already present in neural progenitor cells. A major goal of our work is to identify molecular targets that may lead to new therapeutic strategies for FXS such as targeting the early stages of FXS during embryogenesis.
Cell-based strategies to model human diseases have become a widely accepted complement, or even an alternative, to traditional animal-based disease models - most of which do not accurately phenocopy the targeted human disease. It is especially notable that neural differentiation of hPSCs has recently been shown by multiple groups to temporally and phenotypically recapitulate corticogenesis. In order to establish reliable in vitro phenotypic models for FXS, we have generated a bank of induced pluripotent stem cell (hiPSC) lines from a variety of FXS-affected individuals with varying clinical manifestations of the disease. By differentiating these FXS-hiPSC lines along the neural lineage, we are studying the early neurodevelopmental deficits in FXS using global transcriptome profiling and epigenetic patterning in order to build a molecular interaction map of the early stages of FXS. This will enable us to identify genes and pathways that are dysregulated early in FXS, which are putative targets for future in utero therapies.

F-2123

FIELD POTENTIAL MEASUREMENT USING THE IPS CELL-DERIVED CARDIOMYOCYTES FOR THE PREDICTION OF CARDIOTOXICITY IN VITRO

Mototsugu Sakakibara, **Tetsuo Kitamura**, Makoto Otsuki, Fumihide Bunai, Daisuke Minami, Yukiko Fujisaki, Masaru Sekijima, Tomoharu Osada
Mitsubishi Chemical Medience Corporation, Kamisu, Japan

To provide a better in vitro assay system to predict cardiotoxicity during the early stages of drug development, we are developing a drug screening system based on the multi-electrode array recording using human iPS-derived cardiomyocytes.
We measured the field potential (FP) of iPS-derived cardiomyocytes. And their drug responsiveness was assessed with FP and other indexes acquired from our system. E-4031 and dl-sotalol, which are categorized as IKr blockers, showed prolongation of field potential duration (FPD). Chromanol 293B, categorized as IKs blocker, also showed prolongation of FPD. Diltiazem, categorized as Ca^{2+} -channel blocker, showed curtailment of FPD, and Bay K 8644,

categorized as Ca^{2+} -channel agonist, contrary showed prolongation of FPD. From the data of 13 compounds examined here, working concentrations of them were found for the assessment the FPD prolongation which probably corresponds to QT prolongation. Moreover, combined with FPD, short term variability also contributes to the assessment regarding potential risk of drug-induced arrhythmia, which is exemplified in the case of dl-Sotalol. The data also suggest that this system is applicable to the assessment of Na^{+} -channel blockers under appropriate experimental conditions.
Our data demonstrated that the FP recording under our experimental protocols detected the drug-responsiveness of human iPS-derived cardiomyocytes, which data were mostly reasonable to compare with conventional methods. Our challenge is to enable the risk assessment of unknown compounds with the indexes defined in our FP recording system, that could contribute to efficacy of the exploratory processes of drug development as well as safety pharmacological study.

F-2124

GENOMIC IMPRINTING IS VARIABLY LOST DURING REPROGRAMMING OF MOUSE IPS CELLS

Sachiko Takikawa, Chelsea Ray, Xin Wang, Yulia Shamis, **Xiajun Li**
Department of Developmental & Regenerative Biology, Ichan School of Medicine at Mount Sinai, New York, NY, United States

Derivation of induced pluripotent stem (iPS) cells from somatic cells is a stochastic epigenetic reprogramming process . Genomic imprinting is established in the male and female germ lines in mammals. The epigenetic marks associated with genomic imprinting are inherited in germ cells and they are stably maintained in somatic cells after fertilization. There are some reports and often times contradictory results regarding the status of genomic imprinting in iPS cells derived in different laboratories using different methods and culture conditions. To gain further insight into this epigenetic reprogramming phenomenon, we derived multiple iPS clones from the mouse embryonic fibroblast (MEF) cells that carry the OSKM transgene for inducible expression of four reprogramming factors (*Oct4*, *Sox2*, *Klf4* and *c-Myc*) . After bisulphite mutagenesis, we found that DNA methylation imprint was variably lost at multiple imprinted domains in these iPS clones. Because these MEF cells were generated from the cross between the wild-type DBA/2 female mice and the male mice with the OSKM transgene primarily on a 129 genetic background, we were able to identify SNPs at several imprinting control regions that were different between the maternal and paternal chromosomes. After bisulphite sequencing of genomic DNA samples of these iPS clones, we found that the original DNA methylation imprint present in the parental cells was indeed variably lost at a large subset of imprinted regions among these iPS clones that we isolated from the MEF cells. As a result of loss of genomic DNA methylation imprint in these imprinted regions, the corresponding imprinted genes became bi-allelically expressed based on allele-specific analysis of the RT-PCR product derived from these iPS clones. This loss of parental genomic imprinting in iPS cells could be either caused by the reprogramming process during iPS cell derivation or could be due to instability of genomic imprinting in these iPS clones. To distinguish these two possibilities, we cultured multiple independently derived iPS clones for many generations until P20 (passage 20) and analyzed both DNA methylation imprint and transcripts derived from the corresponding imprinted genes. Our results suggest that loss of genomic imprinting in iPS clones is likely due to the reprogramming process during iPS cell derivation from somatic cells. Furthermore, we found that DNA methylation imprint can be completely erased in iPS cells at multiple imprinted regions. Thus, iPS cell derivation process may be employed to dissect the underlying mechanism of imprinting erasure and resetting in mammals.

F-2125

REPROGRAMMING OF STROMAL-PRIMED HUMAN MYELOID PROGENITORS GENERATES HIPSC WITH HIGH FIDELITY STATES OF PLURIPOTENCY AND AUGMENTED MULTILINEAGE DIFFERENTIATION CAPACITIES

Tea Soon Park[1], Ludovic Zimmerlin[1], Ann Peters[1], Karan Verma[1], Jeffrey Huo[1], Connie C. Talbot[2], Kejin Hu[3], Igor Slukvin[3], Renee Reijo-Pera[4], Elias Zambidis[1]

[A]Institute for Cell Engineering, [B]Institute for Basic Biomedical Science, [1]Johns Hopkins School of Medicine, Baltimore, MD, United States, [2]Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, WI, United States, [3]Stem Cell Biology Program, Stanford University, San Francisco, CA, United States

Conventional nonviral non-integrating reprogramming of human somatic cells to a pluripotent state is slow, inefficient, and generates hiPSC with highly variable differentiation potencies. We have recently described an episome-based reprogramming method to derive iPSC from stromal-primed (SP) myeloid progenitors with remarkably high efficiencies (up to 50% in transgene-expressing donor cells). Here, we show that this system not only increases reprogramming efficiency, but also impacts the intrinsic differentiation potential of SP-myeloid-iPSC. Myeloid progenitors from CD34+ cord blood (CB), fetal liver (FL), or peripheral blood (PB) cells were reprogrammed via nucleofection of four factors (a single EBNA-based plasmid with SOX2, OCT4, KLF4, c-MYC: SOKM) or seven factors (SOKM plus NANOG, SV40T-antigen, and LIN28). Episomal SP-myeloid-iPSC and non-primed (non-SP) myeloid-iPSC TRA-1-81+ clones were selected at 21 days and confirmed transgene-free after 6-8 passages. These hiPSC were analyzed by transcriptomic and differentiation assays and directly compared to hESC and fibroblast derived-iPSC (fibro-iPSC). Q-RT-PCR analysis revealed that SP-myeloid-iPSC (n=4) expressed pluripotency genes (e.g. c-MYC, NANOG, hTERT, KLF4, TP53, DNMT3B, REX1, and UTF1) at levels that were significantly higher than fibro-iPSC (n=5) and comparable or surpassing hESC. Using Illumina microarray analysis of undifferentiated hESC (n=5), SP-myeloid-iPSC (n=15), and fibro-iPSC (n=9), hierarchical clustering analysis revealed that hESC clustered tightly with SP-myeloid iPSC derived from CB and FL clustered indistinguishable from hESC (R^2=0.99) but distinctly from fibro-iPSC (R^2=0.97). Hemato-vascular differentiation capacity was assessed using our established hemogenic endothelial differentiation system. SP-myeloid-iPSC (7F-CB, 4F-CB and 4F-PB) lines produced consistently robust amounts of CD34+CD45+ cells (26±3%, n=14) at significantly higher levels than hESC (6.6±1.2%, n=6), fibro-iPSC (9.2±1.4%, n=8) and non-SP-iPSC (4.5±1.2%, n=10). SP-myeloid-iPSC also generated significantly higher amounts of CD31+ endothelial cells (17.5%±1.8%, n=21) than fibro-iPSC (9.9±1.7%, n=11) and non-SP-iPSC (11.7±3%, n=9), and similar to hESC (15.1%±2.5%, n=14). To uncover potential differentiation bias from retention of hematopoietic donor somatic memory, we investigated the differentiation capacity of these hiPSC to non-hematopoietic lineages. When cultured in neurogenic differentiation conditions, SP-myeloid-iPSC generated over 91% nestin+ cells, a result comparable to hESC (90.7%) and greater than fibro-iPSC (79.5%), which was confirmed by analysis of Pax6 and NCAM1 expression by Q-RT-PCR. Osteogenic differentiation of SP-myeloid-iPSC lines (n=6) was similarly robust, and produced alizarin red+ bone nodules at similar or greater intensities than hESC, and exceeded the poor and highly variable efficiencies of non-SP-myeloid hiPSC (n=3) and fibro-iPSC (n=4). In summary, micro-environmental signals during the initial phases of myeloid progenitor reprogramming was essential to generating hiPSC with multi-lineage differentiation capacities that were comparable or superior to hESC. These data highlight the principle that not only the donor cell type, but also its proper micro-environmental conditioning are critical for obtaining high-fidelity hiPSC with pluripotent differentiation capacities comparable to embryo-derived hESC.

F-2126

IPSC FROM REPOSITORY SPECIMENS: A COMPARISON OF T-CELLS AND LCLS

Jennifer C. Moore[1], Alana Toro-Ramos[2], Michael Sheldon[1], Jay A. Tischfield[1], Ronald P. Hart[1]
[A]Genetics, [B]Cell Biology and Neuroscience, [1]Rutgers University, Piscataway, NJ, United States

The development of methods to derive induced pluripotent stem cells (iPSC) using tissues collected from human subjects represents a potential milestone in the study of disease. The use of blood as a source for iPSC presents several advantages. The drawing of blood, or phlebotomy, is less invasive than the methods used to collect other types of source cells for reprogramming, such as a skin punch biopsy for fibroblasts, increasing the likelihood of subject consent. Institutional Review Boards are also more likely to approve protocols based on phlebotomy. Of potentially great import is that blood cells, in the form of cryopreserved primary lymphocytes (CPLs) or EBV-transformed lymphoblastoid cell lines (LCLs), are the most commonly stored cell type in major repositories such as the RUCDR (www.rucdr.org). These vast existing collections, associated with robust clinical data accumulated over a number

of years or decades, have often been subjected to genome-wide association studies (GWAS), and can be used to select cell lines with carefully defined genotypes and phenotypes. Two non-integrating reprogramming methods are available: infecting T cells enriched from CPLs with Sendai viruses or transforming LCLs with episomal plasmids. Although CD4+ T cells can very efficiently be reprogrammed into iPSC, these cells have a more limited capacity to proliferate and they may not be retained in some repository collections. In these cases it would be convenient to use commonly-stored LCLs. These cells have the advantage of being able to proliferate indefinitely. Although LCLs are less efficiently reprogrammed, the resulting iPSC are comparable to T cell derived iPSC in expression of pluripotent markers by FACS and by large scale gene expression analysis such as the PluriTest. Both source cells can also efficiently differentiated into all three germ layers as measured by an in vitro embryoid body assay. Protocols to enhance the efficiency of LCLs would be valuable, but having parallel methods to reprogram repository cells provides flexibility.

F-2127

FOOT PRINT-FREE AND XENO-FREE GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS

Dong-Youn Hwang
Biomedical Sciences, CHA University, Seongnam, Korea, Republic of

Due to an extraordinary speed of development in the
iPS cells research, we know that iPS cells share many key properties with ESCs
including pluripotency, self-renewal, morphology, colony formation, and gene
expression profiles. These similar traits of iPS cells to ESCs are thought to
lead the cells to clinical applications in the foreseeable future.
Two important safety-related issues for clinical application
of iPS cells is the (1) generation of iPS cells without chromosomal integration
of reprogramming genes and (2) generation and expansion of the cells in the
absence of animal-derived products in the media.
It was reported that hESCs incorporated significant
amount of nonhuman sialic acid, Neu5Gc, in the cell surface proteins which may
result in failure in transplantation due to immune rejection. Furthermore, iPSCs
cultured in animal-derived products can be a source for non-human pathogen
transmission to human. In that sense, conventional mouse embryonic fibroblast (MEF)-based
culture methods are not suitable for generation and culture of iPS cells for
clinical applications.
In this study, we were able to generate footprint-free
iPSCs in a feeder-free & xeno-free culture condition. Our study provides
valuable information which would expedite clinical application of human
pluripotent stem cells to treat many incurable diseases in the near future.
*This
research was supported by Stem Cell Research Program (2010-0020350) funded by
the MEST and Grant A120254-1201-0000200 funded by the Ministry of Health &
Welfare, Republic of Korea.*

F-2128

ELUCIDATING X CHROMOSOME REACTIVATION AND INACTIVATION IN PLURIPOTENT STEM CELLS

Kiichiro Tomoda[1], Masayo Yumoto[2], Karen Leung[2], Kazutoshi Takahashi[3], Aki Okada[3], Megumi Kumazaki[3], Salma Sami[1], Barbara Panning[2], Shinya Yamanaka[1]
[1]The Gladstone Institute, San Francisco, CA, United States, [2]University of California, San Francisco, San Francisco, CA, United States, [3]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

Female human induced pluripotent stem cell (hiPSC) lines exhibit variability in X-inactivation status. The majority of hiPSC lines maintain one transcriptionally active X (Xa) and one inactive X (Xi) chromosome from donor cells. However, at low frequency, hiPSC lines with two Xas are produced, suggesting that epigenetic alterations of the Xi occur sporadically during reprogramming. Recently, we showed that the X-inactivation status in female hiPSC lines depends on derivation conditions. hiPSC lines generated by the Kyoto method (retroviral or episomal reprogramming), which uses leukemia inhibitory factor (LIF)-expressing SNL feeders, frequently had two Xas. Early passage Xa/Xi hiPSC lines generated on non-SNL feeders were converted into Xa/Xa hiPSC lines after several passages on SNL feeders, and supplementation with recombinant (r)LIF caused reactivation of some of X-linked genes even cultured on non-SNL feeders. Thus, LIF plays a role in the Xi-reactivation, but SNL feeders have additional activities, other than LIF production, for frequent reactivation of the Xi in female hiPSCs.
In this study, to elucidate extracellular signals (cytokines) and downstream transcription factors that induce Xi-reactivation in pluripotent stem cells, we utilized mouse epiblast stem cells in which the GFP transgene is integrated into the Xi (X-GFP mEpiSCs). Because of the Xi integration, GFP is silent in X-GFP mEpiSCs cultured in typical EpiSC medium, which contains basic fibroblast growth factor (bFGF) and Activin A. However, when X-GFP mEpiSCs are cultured in a stringently defined mouse embryonic stem (ES) cell medium that contains two chemical inhibitors for GSK3b and MAPKK and rLIF (2i + rLIF), GFP is turned on, concomitant with the Xi-reactivation. Using this system, we observed that SNL-conditioned medium, a human ES medium (Knockout Serum Replacement (KSR) +bFGF) conditioned with SNL feeders, turned on GFP in X-GFP mEpiSCs more efficiently than 2i + rLIF. In contrast, a human ES medium conditioned with mouse embryonic fibroblast (MEF) feeders did not turned on GFP. When we added rLIF into unconditioned human ES medium, the medium containing rLIF turned on GFP as efficiently as the SNL-conditioned medium. We also tried rLIF in MEF-conditioned medium, but, surprisingly, the rLIF-containing MEF-conditioned medium did not efficiently turned on GFP. These observations, with our previous findings in female hiPSCs, indicate that LIF drives Xi-reactivation and MEF feeders produce secreted factor(s) that counteract LIF function on the Xi-reactivation in pluripotent stem cells. We also found transcription factors that are activated by LIF or the secreted factor(s) and may play roles in the Xi-reactivation. Effects of the factors on Xi-reactivation and X-inactivation in female hiPSCs are now under investigation and will be discussed in this meeting.

F-2131

ONCOGENIC PROPERTY OF INDUCED PLURIPOTENT STEM CELLS DERIVED FROM HUMAN HEPG2 CELL LINE

Kung-Kai Kuo
Kaohsiung Medical University, Kaohsiung, Taiwan

Standard protocol for reprogramming of induced pluripotent stem cells (iPS), including delivering four transcription factors (Oct4/Sox2/Klf4/c-Myc) into cells, has a remarkable potential for cell replacement therapies. However, the teratomas formation after iPS transplantation has limited its clinical application because of the oncogenic activity of proto-oncogene c-myc. In this study, we investigated whether or not this standard reprogramming-technique for iPSCs induction could have any oncogenic promoting effects on transformed cells. HepG2 cell line is derived from a well differentiated liver cancer. We generated HepG2-iPS-like cells by introduction of recombinant lentiviruses encoded standard 4 factor and knockdown vector shp53. Stem cell characters were verified by positive immunohistochemistry staining using various stem cell markers (such as alkaline-phosphatase, SSEA-3, SSEA-4, Tra1-60, and Tra1-81). More tumor spheres formation was also noticed as compared with the original HepG2 cells. Interestingly, HepG2 iPS-like cells showed high tumor formation efficacy after xenograft-transplantation into nude mice. We injected a single colony (200 cells /sphere) per one site in three SCID mice. Five out of twelve injection sites developed tumor formation in two months. And the primary culture cells from these tumors continued to carry a similar tumor phenotype and the genetic property. These cells also demonstrated higher protein expressions of Oct4, Klf4, Sox2 and

ABCG2 (MDR related) than the original HepG2 cells. The endogenous Oct4 and Klf4 were overexpressed more than 2-fold and the exogenous gene expressions of Oct4 and Sox2 were still detectable about 2-fold higher than HepG2. However, we did not detect the significant enhanced expression of oncogene c-Myc. They also exhibited more chemo-resistant characteristics when exposed to 5-FU, cisplatin, and doxorubicin. Taken together, our finding suggested that reprogramming technique for iPSCs may have risks of promoting oncogenicity by unknown mechanisms beside c-Myc in transformed cells, which acquired “cancer stem cell” like behavior from this technique.

F-2132

INSULIN RECEPTOR MUTATIONS ALTER CELLULAR METABOLISM IN HUMAN IPS CELLS

Alison Burkart[1], Salvatore Iovino[1], Youn-Kyoung Lee[2], Laura Warren[1], Katelyn J. Hughes[1], Kristina Kriauciunas[1], Chad Cowan[2], C. Ronald Kahn[1], Mary-Elizabeth Patti[1]

[1]Joslin Diabetes Center, Boston, MA, United States, [2]Harvard University, Cambridge, MA, United States

Insulin resistance is associated with altered insulin action and oxidative metabolism and is a key phenotype linked to risk for type 2 diabetes. However, it is unknown whether cellular metabolism defects are primary features of insulin resistance, and thus present in an undifferentiated state, or emerge only with differentiation or additional environmental stimuli. To address this question, we created and characterized induced pluripotent stem (iPS) cells from patients with Donohue syndrome (leprechaunism) and 3 healthy age-matched controls by retroviral expression of *c-MYC*, *SOX2*, *KLF4*, and *OCT4* reprogramming factors. Donohue Syndrome, which is caused by mutations in the insulin receptor gene, is a condition of severe insulin resistance that is accompanied by reduced growth, severe endocrine disorders, and early death. Consistent with genotype, western blot analysis of patient-derived iPS cells demonstrated a 60% reduction of insulin-stimulated phosphorylation of the insulin receptor ($p<0.05$). This was paralleled by reductions in downstream pathway signaling including AKT, ERK, and GSK phosphorylation. Similarly, expression of key genes involved in insulin signaling, as assessed by qRT-PCR, was also decreased in patient-derived iPS cells, including *INSR* (down 40-80%; $p<0.05$), *IRS1* (down ~30%; $P<0.05$), and *AKT1* (down ~30%: $p<0.05$). Additionally, gene expression analysis using Affymetrix PrimeView arrays demonstrated that expression of other proteins within the insulin signaling pathway were also significantly decreased, including *GAB1*, *PIK3CA*, *PIK3CB*, *RAC1*, and several members of the MAPK family (all $q<0.01$). Furthermore, genes regulating glucose metabolism (*GLUT1*, *GLUT4*, *HK1*, and *PKM2*) were also decreased in both patients, as assessed by qRT-PCR (all down ~40%; $p<0.05$). Interestingly, genes regulating mitochondrial metabolism were relatively unchanged, including subunits of electron transport chain complexes, as assessed by both PCR and microarray. This was paralleled by a ~30% decrease in the extracellular acidification rate (ECAR; measure of lactate production) with no change in the oxygen consumption rate (OCR; measure of oxidative phosphorylation) in the patient iPS lines based on analysis with Seahorse XF24 Analyzer. Patient iPS cells also exhibited increased energetic stress, as demonstrated by increased phosphorylation of AMPK (~6 fold increase; $p<0.05$) and its downstream target ACC (~4 fold increase; $p<0.05$). Direct assessment of reactive oxygen species (ROS) by CM-H_2DCFDA staining revealed increased ROS levels, which can activate AMPK, in patient-derived lines. Additionally, mRNA expression of antioxidant enzymes catalase and *GPX3* was decreased by >60% and 25%, respectively, in both insulin-resistant lines ($p<0.05$). Together, these data indicate that genetically determined impairments in insulin signaling increase oxidative and energetic stress in iPS cells, suggesting that insulin resistance can promote dysregulated cellular metabolism even in the undifferentiated state.

F-2133

DERIVATION AND CHARACTERISATION OF ENDODERM PROGENITORS FROM INTEGRATION-FREE EPISOMAL PLASMID BASED-INDUCED PLURIPOTENT STEM CELLS GENERATED FROM HUMAN FETAL FORESKIN FIBROBLASTS

Peggy Matz[1], James Adjaye[2]

[1]Lehrach, MPI Molecular Genetics, Berlin, Germany, [2]Institute for Stem Cell Research and Regenerative Medicine, Heinrich Heine University, Düsseldorf, Germany

Human embryonic stem cells (hESCs) have two fundamental characteristics. First is pluripotency, i.e. the ability to differentiate to all cell types of the three germ layers endoderm, ectoderm and mesoderm in vitro (formation of embryoid bodies) and in vivo (teratoma formation in immunodeficient mice) Second, hESCs have the capability to self-renew indefinitely. Embryonic stem cells express pluripotency associated markers such as OCT4, NANOG and SOX2 also the surface markers SSEA-4, TRA-l-60, TRA-1-81 and TRA-2-49 but not SSEA-1.
Induced pluripotent stem cells (iPSCs) are embryonic-like cells and can be generated from somatic cells derived from individuals with known genetic characteristics by the over-expression of OCT4 and SOX2 in combination with either KLF4 and c-MYC or NANOG and LIN28.
We have generated episomal-derived and integration-free E-iPSCs from human fetal foreskin fibroblast cells (HFF1) as described by Yu et al. (2009) and compared the transcriptome to that of retro-viral derived HFF1-cells (V-iPSCs) generated in our laboratory. The transcriptome of E-iPSCs are closer to that of hESCs (R2 = 0.9363) in comparison to V-iPSC (R2 = 0.8176). This viral-free method has the advantage over viral-based protocols because of the lack of integrations which otherwise leads to chromosomal re-arrangements of the host genome. Using the E-iPSC line we have derived and characterized hepatocyte-like cells (HLCs) as described by Sullivan et al. (2010) and endodermal progenitors (EPs) as described by Cheng et al. (2012). We detected in HLCs the expression of AFP, Albumin, HNF4α, E-Cadherin and Glycogen. In EPs we detected the expression of LGR5, CXCR4, ALCAM, Albumin, E-Cadherin and HNF4α.
Further studies are planned involving the use of the E-iPSCs derived EPs to generate hepatocyte and pancreatic cells. These studies will enable uncovering the genes and associated pathways that specify a bipotential EP to differentiate to either liver or pancreas. Additionally, these E-iPSCs and derived EPs provide unique resources for disease modeling, developmental studies, drug screening and toxicology studies.

F-2134

MODELING DIAMOND BLACKFAN ANEMIA: REPROGRAMMING IPS CELLS FROM DBA PATIENT FIBROBLASTS

Elizabeth R. Macari[1], Alison M. Taylor[1], Jessica M. Humphries[1], Natasha Arora[1], Yuin Han Loh[1], Alexander L. Devine[1], Andrew Ettenger[1], Kelly Fitzgerald[1], Philip D. Manos[1], Anupama Narla[4], Blanche P. Alter[5], Hanna T. Gazda[2], Colin Stieff[4], Suneet Agarwal[3], Alan Beggs[6], Benjamin L. Ebert[7], Thorsten Schlaeger[1], George Q. Daley[1], Leonard I. Zon[1]

[A]Stem Cell Program, [B]Division of Genetics and Program in Genomics, [1]Children's Hospital Boston, Boston, MA, United States, [2]Division of Hematology/Oncology, Children's Hospital Boston and Dana Farber Cancer Institute, Boston, MA, United States, [3]Department of Health and Human Services, Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD, United States, [4]Division of Genetics and Program in Genomics, Boston Children's Hospital, Boston, MA, United States, [5]Brigham and Women's Hospital, Boston, MA, United States

Diamond Blackfan anemia (DBA) is a rare red cell aplasia that presents in early childhood. Ribosomal protein mutations occur in over 50% of DBA patients. Isolating patient hematopoietic cells for research is difficult, and the animal models that harbor mutations in ribosomal protein genes have not accurately reproduced the disease. The development of patient-derived induced pluripotent stem (iPS) cells represents a critical *in vitro* disease model. We generated iPS cell lines from DBA patient fibroblasts using retroviral, episomal, and lentiviral reprogramming approaches. We currently have nine lines validated by expression of the stem cell markers SSEA-4, TRA160, TRA181, OCT4, and NANOG. These lines also all have a normal karyotype. Six lines are derived from patients with an RPS19 mutation, two lines are from a patient with an RPL5 mutation, and one line is from a patient with a mutation in RPS24. We plan to generate at least 27 lines from patients with mutations in *RPS19, RPL5, RPL11,* and *RPS24,* and will perform protein replacement via lentiviral gene transfer or TALEN-mediated genome editing. Generating

multiple lines from each patient via 3 different reprogramming methods, and having internally controlled, gene repaired cell lines, ensures that any cellular phenotypes are ribosomal protein mutation specific and not due to the reprogramming process. DBA lines support multi-lineage hematopoietic differentiation in methylcellulose and erythroid-specific differentiation of $CD71^+$ erythroid cells. We have observed cell cycle arrest in some DBA lines when re-differentiated into fibroblasts, and are currently investigating the mechanism. iPS cell lines from DBA patients will be valuable tools to study disease pathology and to screen for new therapies directly in DBA patient cells.

F-2135

HIGH RESOLUTION ANALYSIS WITH NOVEL CELL SURFACE MARKERS REVEALS STEPWISE PROGRESSION OF REPROGRAMMING

James O'Malley[1], Stavroula Skylaki[1], Eleni Chantzoura[1], Tyson Reutz[1], Kumiko Iwabuchi[1], Sara Brightwell[1], Anna Johnsson[2], Simon R. Tomlinson[1], Sten Linnarsson[2], Keisuke Kaji[1]
[1]MRC Centre for Regenerative Medicine, Edinburgh, United Kingdom, [2]Karolinska Institute, Stockholm, Sweden

Identification of the important molecular mechanisms of reprogramming is prevented by the low efficiency and heterogeneity of iPS cell generation. Previous studies have demonstrated that down-regulation of the fibroblast-associated marker, Thy-1, and a mesenchymal-to-epithelial transition is followed by up-regulation of the undifferentiated ES cell marker, SSEA-1, and the subsequent expression of various pluripotency genes, such as Nanog. This clearly indicates that reprogramming progress in a stepwise manner. However, to date there has been a lack of detailed information outlining how differentiated cells reach the pluripotent state, and it is still an open question as to whether cells reverse differentiation processes or if other routes are taken. Here we demonstrate a detailed reprogramming route map from mouse embryonic fibroblasts to iPSCs, using two cell surface markers, CD44 and ICAM1, and a Nanog-GFP reporter. Flow cytometry analysis could be used to trace the progression of cells from one stage of reprogramming to the next. RNA-sequencing analysis of intermediate subpopulations isolated with these markers demonstrated that pluripotency genes could be classified as those with either early or late up-regulation during reprogramming. Furthermore, this analysis clearly uncovered that reprogramming entails not merely loss of fibroblastic genes and gain of pluripotency genes, but also involves transient up-regulation of several hundred genes which are neither expressed in fibroblast nor pluripotent cells. Among these transiently expressed genes, gene ontology analysis identified enrichment of epidermis-related genes, indicating reprogramming is not simply the reversal of normal developmental processes. Interestingly, suppression of these epidermis genes resulted in changes to the efficiency and kinetics of reprogramming which could be monitored using our marker system. This finding and marker system will open a new avenue towards better understanding of the molecular mechanisms of reprogramming and improve reprogramming technology through detailed observation of the dynamics of iPS cell generation.

F-2136

COORDINATION OF CELL-CELL INTERACTIONS AND CELL-SUBSTRATE INTERACTIONS BY ALTERING MIGRATION IS REQUIRED FOR REGULATING FATE DECISION OF HUMAN INDUCED PLURIPOTENT STEM CELLS

Mee-Hae Kim, Masahiro Kino-oka
Osaka Univ, Suita, Japan

Understanding fundamental mechanisms that govern cell fate determination of human induced pluripotent stem cells (hiPSCs) provides key strategies to maintain their undifferentiated state during cell expansion. In order to understand the basic mechanisms controlling early cell fate decisions during hiPSC culture, we used dendrimer surfaces, that is, first-generation (G1), third-generation (G3) and fifth-generation (G5) surfaces with SNL feeder cells. When hiPSCs were cultured on G1 surface, cells formed flattened colonies that appear as single-cell layers, and they were gradually smaller, more tightly packed. As the generation number of the dendrimer increased, the cells

on G3 surface exhibited dispersed colonies through active migration, accompanied by cell morphological changes of stretching and contracting, and the integrin-induced tyrosine phosphorylation of paxillin was promoted. Further increment of cell migration was significant on the G5 surface and this phenomenon was thought to be lead formation of concentric ring-like aggregated cells through division and coalescence between cells, and the phosphorylation of paxillin was suppressed. It was found that this morphological change through active migration that occurred on the modified surface with the varied generation numbers of dendrimer was responsible for the correlation between Rac1 and E-cadherin expression, thereby modulating cell fate decision such as survival/death, self-renewal and pluripotent state. These results indicate that this migrative property of hiPSCs can be a useful tool for controlling the colony formation of hiPSC and then govern their self-renewal and fate.

F-2137

INTEGRATION-FREE HUMAN INDUCED PLURIPOTENT STEM CELLS REPROGRAMMING ON HUMAN PLACENTA-DERIVED MSC-FEEDERS AND EVALUATING HIPSCS POTENTIAL FOR TREATMENT OF SPINAL CORD INJURY IN A SCID MICE MODEL

Vikram Sabapathy[1], Durai Murugan[2], Rekha Samuel[1], George Tharion[2], Sanjay Kumar[1]
[1]Centre for Stem Cell Research, Vellore, India, [2]Department of Physical Medicine and Rehabilitation, Christian Medical College, Vellore, India

Human induced pluripotent stem cells (hiPSCs) are generally derived from forced expression of Oct4, Sox2, Klf4, & C-Myc transcription factors in the human somatic cells plated on MEFs (Mouse embryonic feeders). However, the reprogramming efficiency is very low. Further, use of MEFs for generation of hiPSCs makes them impractical for clinical applications. In this project, we have efficiently reprogrammed human placenta-derived MSCs (PD-MSC) to hiPSCs, using virus-free nucleofection protocol by combination of only three transcription factors on autologous human MSC feeders. In addition, we observed that supplementation of 2i inhibitor and LIF further increased the reprogramming efficiency. In-order to analyze the reprogramming process, derived cells were then subjected to various characterization techniques such as immunostaining, Q-PCR, epigenetic analysis, in vitro differentiation assays, embryoid bodies formation etc. When subjected to intramuscular injection in scid mice, the derived iPSCs were capable of forming teratoma like cell mass. Spinal cord injury is serious debilitating disorder that results in the functional loss of motor and sensory neurons below the injury site. We have optimized the conditions for creating spinal cord injury, transplantation of human derived cells and post-operative care in a scid mice model. Our preliminary results with transplantation of human PD-MSCs indicate that transplanted cells efficiently augment lower thoracic spinal injury in SCID mice model. In the next set of experiments, we are transplanting hiPSC-derived neurospheres to evaluate therapeutic efficacy in Spinal cord injury.

F-2138

SMALL MOLECULE CULTURE ADDITIVES ENABLE INDUSTRIAL AND THERAPEUTIC USE OF HIPSCS BY INDUCING NAIVE-LIKE PROPERTIES

Bahram Valamehr, Megan Robinson, Ramzey Abujarour, Betsy Rezner, Florin Vranceanu, Thuy Le, David Robbins, Tom Lee, Peter Flynn
Fate Therapeutics Inc., San Diego, CA, United States

The effective use of human induced pluripotent stem cells (hiPSCs) in drug screening and cell therapy applications would be greatly enhanced by culture systems that allow rapid expansion of homogeneous, pluripotent, genomically stable cells. We have previously described a multiplex platform for the efficient induction, selection and characterization of hiPSCs, enabling rapid and simultaneous production of many qualified hiPSC lines by a single researcher. At the heart of this process is a culture system (SMC4^{TM}), containing a unique combination and concentration of four small molecule inhibitors, that are seen to augment somatic cell reprogramming as well as support the viability, proliferation and self-renewal of established hiPSCs in continuous feeder free and single cell culture. The hiPSCs

generated using this platform uniformly exhibit pluripotency markers such as OCT4 and NANOG as well as readily differentiate into the three germ layers. In addition to these fundamental pluripotent stem cell properties, SMC4^{TM} derived hiPSCs exhibit unique features that are desirable in industrial applications including efficient and scalable expansion on feeder free surfaces, genomic stability and homogeneous monolineage directed differentiation. Interestingly, these characteristics parallel some of those seen in the recently described naïve pluripotent state of hiPSCs. To further investigate the undifferentiated state of SMC4^{TM} hiPSCs, we compared hiPSC clones generated in either conventional medium or SMC4^{TM} supplemented medium. Global gene expression revealed that although both cultured hiPSCs were more similar to each other as compared to their original starting population, a significant number of genes displayed greater than 4-fold expression differences between the two conditions. The majority of these genes are known to be involved in cellular differentiation, suggesting that SMC4^{TM} culture system prevents spontaneous differentiation under pluripotent culture conditions while allowing synchronized differentiation when directed. Xist was significantly repressed in hiPSCs cultured in SMC4^{TM} resulting in the overall increase of X chromosome gene expression; indicating reactivation of the silenced X chromosome. Further, hiPSCs generated and cryopreserved in the SMC4^{TM} culture system demonstrate enhanced post thaw survival and culture quality – removing the usual requirement for for manual clean-up of the culture. The combination of efficient recovery post thaw coupled with robust expansion of SMC4^{TM} generated hiPSCs that are genomically stable will play a critical role in reproducibility and pluripotent culture expansion required for industrial use of hiPSC in large drug screening campaigns and cell therapy manufacturing. hiPSCs cultured in SMC4^{TM} also display a high rate of cloning efficiency and can be single cell sorted at dilute concentrations including 1 cell per well of a 96-well plate; readily enabling genetic modifications at the single cell level for therapeutic purposes including disease correction. Collectively, our findings demonstrate that SMC4^{TM} generated hiPSCs' unique naïve-like properties suggest this culture system is ideal for industrial applications.

F-2141

INDUCED PLURIPOTENT STEM CELL MODELING OF MULTISYSTEMIC, HEREDITARY TRANSTHYRETIN AMYLOIDOSIS

Amy Leung[1], Shirley K. Nah[1], Gustavo Mostoslavsky[1], Darrell N. Kotton[1], David C. Seldin[2], George J. Murphy[1]
[A]Boston University Center for Regenerative Medicine (CReM), [B]Amyloidosis Center, [1]Boston Univ School of Medicine, Boston, MA, United States

Familial transthyretin amyloidosis (ATTR) is a lethal, autosomal dominant protein folding disorder caused by more than 100 distinct mutations in the transthyretin (TTR) gene. Mutated transthyretin, a transport protein predominantly produced and secreted by the liver, circulates in the bloodstream, aggregating and depositing primarily in the peripheral nervous system and the heart. While aberrant TTR is produced and secreted by the liver, disease-characteristic cellular damage is seen peripherally, primarily in the nervous system and in cardiac tissue. Insights into the mechanisms of these disease features and development of innovative therapeutics have been limited by the lack of a mouse model and the inability to study ATTR in the appropriate human cell context and genetic background.
The generation of induced pluripotent stem cells (iPSC) through the reprogramming of somatic cells from patients with inherited diseases provides an unprecedented opportunity to study the effects of genetic abnormalities and disease progression. The derivation of unlimited quantities of the genetically relevant cell types targeted and affected in patients allows investigation of the cellular, molecular, and epigenetic events involved in a multisystem, genetic disease such as ATTR. Although most cases of ATTR are due to a single base pair mutation predominantly expressed in the liver, end organ damage occurs outside the liver, highlighting the need for a model capable of recapitulating the multisystem complexity of the disease.
Using iPSC technology, cell lines can be established that are genetically identical to the individual from whom they are derived, allowing for disease modeling and development of novel therapeutics in the exact genetic context of the patient. Here, we have generated the first known disease-specific iPSC lines from a patient with ATTR. Harnessing the pluripotency of iPSC, we demonstrate the modeling of this multisystem disease through the directed differentiation of patient-specific iPSC into hepatocytes that produce mutant TTR protein as well as cardiomyocytes

and neurons normally targeted in ATTR. We demonstrate that ATTR iPSC-derived neuronal and cardiac cells display oxidative stress and an increased level of cell death when exposed to mutant TTR protein produced by the patient's own iPSC-derived hepatocytes, recapitulating essential aspects of the disease in vitro. Furthermore, small molecule stabilizers of TTR that are being tested in clinical trials show efficacy in this model, ameliorating the damaging effects of ATTR exposure and validating this iPSC-based, patient-specific in vitro system as a platform for testing therapeutic strategies. To date, iPSC-cell based models have been utilized to model genetic diseases in a single lineage in which the variant protein functions, and to our knowledge, this is the first example of the use of iPSC technology to model a multisystem disease in which the effects of variant protein produced by one organ manifests disease in other target tissues. Our work documents the successful modeling of ATTR in vitro with the use of iPSC technology, demonstrating that it is possible to model a long-term, complex, multisystem disease in a relatively short space of time, using lineage-specified cells derived from patient-specific stem cells.

F-2142

DIFFERENTIAL REPROGRAMMING EFFICIENCY OF HUMAN ADIPOSE TISSUE-DERIVED CELLS IN RETROVIRUS-MEDIATED IPS INDUCTION

Seok-Jin Kang[1], Young-Il Park[1], Sung-Won Park[1], Hyo-Shuk Shin[1], Yool-Hee Yang[2], Sa-Ik Bang[3], Seong-Wan Son[1], Hwan-Goo Kang[1]

[1]Toxicology & residue chemistry division, Animal, plant and fisheries quarantine and inspection agency, Anyang-si, Gyeonggi-do, Korea, Republic of, [2]Samsung Biomedical Research Institute, Samsung Medical Center, Seoul, Korea, Republic of, [3]Department of Plastic Surgery, School of Medicine, Sungkyunkwan University, Seoul, Korea, Republic of

Human adipose tissue is comprised of high quantity of mesenchymal stem cells (MSCs) and easily obtainable. Therefore, adipose tissue-derived MSCs were reported as a valuable cell source for inducing the induced pluripotent stem cells (iPS) and cell therapy.
In this study, adipose tissue-derived cells were surgically collected from five patients. Adipose tissue derived-MSCs were sorted by MACS separation using CD105 antibody, a representative MSC marker. The ratio of CD105+ cells was different from tissue origins (abdomen vs. breast; 68.1% vs. 10.9%) regardless of patient's age and gender. For characterization of adipose tissue-derived cells using surface markers (CD13, CD29, CD34, CD44, CD45 and CD105), each adipose tissue-originated CD105+ cells appeared typical phenotype of MSCs while CD105- cells represented a flat fibroblast-like cells containing a minor portion of CD105+ cells (21.9%).
For iPS induction, CD105+ (p4) and CD105- cells (p3) were transduced by retroviral vector containing four reprogramming factors (OCT4, SOX2, KLF4 and C-MYC). During the reprogramming process, the reprogramming efficiency of CD105+ and CD105- cells were 0.009% and 0.016% with TRA-1-60 expression and 0.025% and 0.048% with AP staining at day 20 of post-transduction, respectively. Also, the reprogramming of CD105+ cells was likely to be more retarded than CD105- cells as compared with size of TRA-1-60+ colony (0.57×10^5 vs. 1.25×10^5 μm^2).
We hypothesized that retroviral infectivity could result in the lower efficiency and retardation in CD105+ cells. Therefore, retroviral infectivity and transgene amount were indirectly measured by control vector (pMXs-IRES-GFP) for examination of this result. Interestingly, viral infectivity was very low at CD105+ cells (NIH3T3 vs. CD105- vs. CD105+; 87.7% vs. 63.5% vs. 9.9%). The GFP amounts of CD105+ cells were 47.3% against CD105- cells. Additionally, quantities of four reprogramming factors were from 22.5 to 34.5% against CD105- cells.
In conclusion, adipose tissue-derived CD105+ cells showed relatively lower reprogramming efficiency and more delayed colonization than CD105- cells. It could be inferred that CD105+ cells are a hard cell type to delivery transgene by retroviral vector system.

F-2143

REPROGRAMMING AND CULTURE OF HUMAN INDUCED PLURIPOTENT STEM CELLS ON DISHES GRAFTED WITH CELL ADHESION PEPTIDES

Jia-Yi Lin, Ta-Chun Kao, Meng-Hsueh Wu, Akon Higuchi
Department of Chemical Engineering and Material Sciences, National Central University, Jhongli, Taiwan

Human induced pluripotent stem cells (hiPSCs) have significant potential in therapeutic applications for many diseases because they have the specific ability to differentiate into all types of human somatic cells. However, the tentative clinical potential of hiPSCs is restricted by the use of mouse embryonic fibroblasts (MEFs) as a feeder layer. The feeder-free cultures using synthetic biomaterials having nanosegments as stem cell culture materials offer more reproducible culture conditions and lower the cost of production without introducing xenogenic contaminants. These improvements will increase the potential clinical applications of differentiated hiPSCs. Here we report that hiPSCs can be successively generated without usage of a feeder layer of MEFs during generation of hiPSCs by transfection of retrovirus containing pluripotent genes into human adipose-derived stem cells (hADSCs) where hiPSCs were cultured on polyvinylalcohol-co-itaconic acid (PVA-IA) grafted with several nanosegments (KGGPQVTRGDVFTMP [cell-binding domain derived from vitronectin, oligoVN], KGGNGEPRGDTYRAY [cell-binding domain from bone sialoprotein, oligoBSP], and GKKQRFRHRNRKG [heparin-binding domain, oligoHBD]). The elasticity of PVA-IA dishes grafted with nanosegments was regulated from 100 kPa to 16,000kPa by control of crosslinking time of PVA-IA. At day 4 after transfection, hADSCs transfected with pluripotent genes (Oct4, Sox2, Klf4, and c-Myc) were shifted to be cultured on MEFs as control experiments and on PVA-IA dishes grafted with nanosegments. hiPSC colonies were clearly observed for the cells cultured on MEFs at day 14 after transfection, while hiPSC colonies were clearly detected on dishes grated with oligoVN and oligoBSP having elasticity from 100kPa to 16,000kPa after the passage of the cells. The number of colonies generated on MEFs was 120±28 per dishes, while that generated on VN-dishes was 20-80 per dishes when 105 hADSCs were seeded on the dishes. It was found that the efficiency of hiPSC generation on the VN-dish at feeder-free conditions was less than that on MEFs. However, the hiPSC colony showed alkali phosphatase activity much clearly, and immunohistochemistry suggested that the hiPSCs were generated on PVA-IA dishes grafted with oligoVN and oligoBSP expressing pluripotent protein of SSEA-4 at feeder-free conditions. hiPSCs prepared on MEFs as well as PVA-IA dishes having nanosegments generated teratoma and embryonic bodies containing different cell types of the three germ layers, which suggest hiPSCs cultured on PVA-IA dishes having nanosegments keep their pluripotency and can differentiate into the cells of three germ layers. There are several reports for the culture of hiPSCs on feeder-free conditions. However, hiPSCs were generated and cultured on MEFs at first before the culture of hiPSCs on feeder-free conditions in most of cases. This study reports that hiPSCs have been generated on synthetic dishes (PVA-IA dishes grafted with nanosegments) at feeder-free culture. It was found that the optimal elasticity (1000kPa to 10,000kPa) and specific nanosegments (oligoVN and oligoBSP) of the cell culture dishes improve to keep pluripotency of hiPSCs on the dishes where hiPSCs were cultured.

F-2144

CHARACTERISTIC OF HUMAN INDUCED PLURIPOTENT STEM CELLS DERIVED FROM DIFFERENT CELL ORIGINS UNDER FEEDER-FREE CONDITIONS

Ta-Chun Kao, Jia-Yi Lin, Meng-Hsueh Wu, Akon Higuchi
Department of Chemical Engineering and Material Sciences, National Central University, Jhongli, Taiwan

Human induced pluripotent stem (hiPS) cells have become an important cell source for regenerative medicine, tissue engineering, and disease modeling. hiPS cells promise to solve issues associated with usage of embryonic stem (ES) cells in clinical applications, such as immune responses from the patient and the ethnic issues regarding the use of human embryos. hiPS cells open avenues for the cell source of personalized disease models on drug treatment evaluation and analysis of disease mechanism. The generation of iPS cells has been extensively studied using several cell sources, which are especially obtained by noninvasive method, under feeder-free and xeno-free conditions. Blood cells and adipose tissue-derived cells might be the most obtainable cell source to generate hiPS cells. However, little is known about the cellular characteristics of hiPS cells and the role of physical and chemical cues of biomaterials where the cells are reprogrammed and hiPS cells are cultured and differentiated into specific lineages of the cells. Previous studies have shown that controlled lineage specification of mesenchymal stem cells (MSCs) can be achieved via manipulating the elasticity of matrix material or through small functional groups or ex-

tracellular matrixes (ECMs) immobilization on the cell culture material surfaces. We found that MSCs derived from human adipose tissue (ADSCs) and human amniotic fluid (AFSCs) responded differently to physical cues, such as matrix elasticity, of the cell culture materials. In this study, we hypothesized that hiPS cell fate will be affected by their physical characteristics (e.g., elasticity) of cell culture materials and that hiPS cells derived from different cell source may respond to the physical cues of cell culture materials differently depending on their origin. We are evaluating that hiPS cells might be less pluripotency than ES cells as is previously believed, and that hiPS cells retain the original characteristics of their parental cells even after reprogramming of the cells with transfection of pluripotent genes. Here, we generated hiPS cells from various cell sources including human adipose tissue, human amniotic fluid, human umbilical cord, human umbilical cord blood, and human peripheral blood using feeder-free and xeno-free conditions and cultured on biomaterials grafted with several oligopeptides.

F-2145

CULTURE ENVIRONMENT ON THE DIFFERENTIATION OF NEURONS ORIGINATING FROM HUMAN IPS CELLS

Banri Yamanoha
Soka Univ, Faculty of Engineering, Tokyo, Japan

Human induced pluripotent stem (hiPS: 253G1, 201B7) cells can differentiate into human neural stem cells by performing a multi-stage culture (S.Yamanaka et al.). Neural stem cell is a cell having the capability of both self-renewal and multipotency, terminally differentiated cells become neurons, astrocytes and oligodendrocytes via progenitor cells. Possible applications of human pluripotent stem cells, advantage is born by using human-derived cells. More specifically, it is used for the purposes of regenerative medicine and pharmaceutical and chemical assessment. In particular, the use of neural cells derived from human cells can be achieved.
This presentation, we focus on the culture conditions to differentiate into different types of neurons from hiPS. HiPS cell in culture conditions has the property to form colonies while cell proliferation. In order to obtain the desired differentiated cells, this hiPS colony is essential for maintaining undifferentiated. For adjusting the feeder cell layer of human fibroblasts, human fibroblasts were prepared from hiPS cells. In particular, the creation of human fibroblasts was performed in human-derived reagents. Human fibroblasts grown in 10-cm dishes were inactivated in Mitomycin C solution and isolated. Inactivated fibroblast was stored in frozen till used. Undifferentiated iPS colonies were obtained by co-cultured with human fibroblast.
We were carried out sequentially processing method known as a NSS method using ES cells by Nakayama and Inoue, 2004. Undifferentiated colonies were isolated from co-culture dish. The isolated colonies were cultured in suspension culture dish containing FGF-2 (basic fibroblast growth factor) and the ACM (astrocyte conditions medium). It was processed to prevent cell adhesion. Colonies were cultured in suspension were differentiated into neural stem spheres. Astrocytes were differentiated from hiPS cells also. After incubation of the human astrocytes, recovered a culture medium and it was stored until use. The culture dish was coated with substrates for cell adhesion in advance, and neural stem sphere was transferred on the culture dishes. The single cell had migrated toward the outside from edge of the spheres after a few days later. Then, the cells were harvested and seeded into new adherent culture dish.
Identifications of differentiated neural cells and undifferentiated HiPS cells were determined by immunofluorescence staining and real-time PCR. The degree of undifferentiated was used for Oct3 / 4, the alkaline phosphatase and Nanog. Neuronal cells in differentiated was evaluated using followings antibodies Nestin, A2B5, GFAP, MAP-2, O4. Neural differentiations were assessed using the respective antibodies GABA, Ach, Glutamine, Dopamine.
As described above, in this experiment we have carried out the identification of neurons and progenitor cells under a human-derived cell culture conditions.

F-2146

APPLICATION OF EPIGENETIC SWITCH FOR GENERATION OF HUMAN IPS CELLS.

Joanna Wroblewska[1], Katarzyna Kulcenty[1], Urszula Oleksiewicz[1], Wiktoria Suchorska[2], Jannik Andersen[3], Giulio Draetta[3], Gustavo Mostoslavsky[4], Maciej Wiznerowicz[1]

[1]Dept of Cancer Diagnostics and Immunology, Gene Therapy Laboratory, Poznan University of Medical Sciences, Poznan, Poland, [2]Radiobiology Laboratory, Greater Poland Cancer Centre, Poznan, Poland, [3]Institute for Applied Cancer Science, University of Texas MD Anderson Cancer Center, Texas, TX, United States, [4]Dept. of Medicine, Boston University School of Medicine, Boston, MA, United States

Human induced pluripotent stem cells (IPS) are generated by reprogramming of somatic cells through enforced expression of embryonic transcription factors. However, clinical applications require that expression of introduced transgenes must be permanently switched off in the IPS cells and obtained differentiated progenies.
Here, we took advantage of epigenetic switch that relies on reversible binding of tTRKRAB transrepressor to tetO element, which results in tight transcriptional repression of proximal promoter through heterochromatin formation.
In order to apply this system for reprogramming, the tetO element was inserted into pSTEMCCA lentiviral vector carrying OCT4, SOX2, KLF4 and cMYC under control of EF-1alpha promoter. Transduction of human skin fibroblasts with obtained pSTEMCCA-tetO allowed for expression of reprogramming factors and thus efficient generation of human iPS clones. Obtained clones were picked and further cultured until establishing stable IPS cell lines. Then cells were then transduced with lentiviral vector pLV-HK carrying tTRKRAB, in order to switch off reprogramming transgene expression.
Tight repression of introduced transgenes in all human IPS clones was analyzed by RT-PCR and confirmed full functionality of our system. Obtained IPS cell lines showed no abnormalities in karyotypes. Pluripotent phenotype of IPS cells was revealed by analysis of endogenous embryonic genes expression using RT-PCR and immunofluorescence staining. Analyzed cells were also able to form embryonic bodies in vitro and teratomas in immunocompromised mice, which proved their ability to differentiate into cells derived from three germ layers. tTRKRAB-mediated epigenetic repression persisted through prolonged culture of obtained IPS cell lines. Importantly, expression of introduced transgenes remained undetectable after differentiation into embryonic bodies. In order to confirm molecular homogeneity of obtained IPS cell lines, high throughput molecular profiling including RNA-Seq and global DNA methylation analysis are currently being performed.
Our results confirm that our epigenetic switch effectively prohibits re-expression of embryonic transgenes in human IPS cells and their differentiated progenies paving the way for their applications in various fields of regenerative medicine, disease modeling and drug discovery.

F-2147

AN ALTERNATIVE APPROACH TO PREDICTING SKELETAL TERATOGENICITY WITH PLURIPOTENT STEM CELLS: COMPARISON OF HESCS AND HIPSCS

Nicole R.L. Sparks[1], Ivann K.C. Martinez[1], Bir Bhanu[2], Nicole I. zur Nieden[1]

[A]Department of Cell Biology, Neuroscience and Stem Cell, College of Natural and Agricultural Sciences, [B]Center for Research in Intelligent Systems, Bourns College of Engineering, [1]University of California Riverside, Riverside, CA, United States

The embryonic development of an organism represents a relatively short, yet vulnerable, span in its entire life cycle. Therefore, any teratogenic compound acting upon the developing organism during pregnancy may severely perturb development. In the early 1960's, the thalidomide disaster established the need for studies on developmental toxicity when pregnant women taking the drug and doctors discovered the teratogenicity of thalidomide. For the first time, this demonstrated the urgent need to identify and predict the developmental toxicity of putative toxicants prior to being released into the environment. Today, human and animal populations are experiencing a rapid increase in exposure to potential environmental toxicants in the form of pharmaceutical drugs, commercial chemicals, industrial by-products, wastes and a number of drugs not properly tested. In the United States, over 1500 new compounds enter the environment each year and all have a potential to cause musculoskeletal abnormalities.

Apart from the tremendous associated costs, *in vivo* testing of such a magnitude of chemicals would require enormous amounts of animals and a great deal of time. Clearly, *in vivo* testing methods would not be feasible to adequately test all current and putative substances. Hence, it is crucial to develop alternative *in vitro* methods to assess toxicity, especially teratogenicity, to test current and putative chemicals for their impact on prenatal development while at the same time reduce the use of animals and cut expenses. For instance, utilizing a human *in vitro* model based on embryonic stem cells (hESCs) will provide a reliable screening assay of developmental toxicity and abrogate the use of animal models. Though versatile, the use of hESCs brings forth ethical challenges. To improve *in vitro* developmental toxicity screening, we propose that utilization of human induced pluripotent stem cells (hiPSCs) will replace the challenges of hESCs and limit the reliance on animal models.
In order to compare their ability to predict skeletal teratogens, hESCs and hiPSCs were first induced to differentiate into osteoblasts to evaluate their respective osteogenic differentiation potential. Osteogenic differentiation was achieved through an over-growth approach with the addition of an osteogenic cocktail, 10 mM ß-glycerophosphate, 50 µg/ml ascorbic acid, and 5×10^{-8} M $1\alpha,25\text{-}(OH)_2$ vitamin D_3. Molecular analysis of stage-specific bone marker gene expression, calcium content, and alkaline phosphatase activity illustrated that hiPSCs do differentiate into osteoblasts, similar to hESCs. However, differences in differentiation kinetics and yield were identified. Despite these differences, MTT analysis demonstrated that hiPSCs do posses a teratogenic sensitivity to the test compound Fluorouracil (5-FU), a strong teratogen.
In conclusion, hiPSCs are an effective model to investigate skeletal teratogenicity of environmental chemicals. This alternative approach will eliminate species-species variability, common in animal models, as well as replace *in vivo* testing to reduce animal sacrifices. More importantly, hiPSCs will not endure the ethical or political challenges of hESCs.

F-2148

INDIVIDUAL PLURIPOTENT STEM CELL CLONES RECAPITULATED DISTINCT STAGES IN ERYTHROPOIESIS BY SELF-REPLICABLE ERYTHROCYTE PROGENITORS

Kiyosumi Ochi, Sou Nakamura, Takeaki Dohda, Naoya Takayama, **Koji Eto**
The Center for iPS Cell Research and Application (CiRA), Kyoto, Japan

Human induced pluripotent stem cells (hiPSCs), for example established from Rh-minus O ABO blood type donor, are potentially inexhaustible sources for transplantation by erythrocytes. But how huge number (1012 per patient in transfusion) of erythrocytes can be generated in vitro is still obstacle. Another point is how higher efficiency of enucleation is achieved by obtaining mature stage of erythrocyte progenitors.
In solution, we have previously proposed that a self-replicable erythrocyte progenitors (erythroblasts) as a material source for transplantable erythrocytes, in which gene manipulation with c-MYC and BCL-XL into hematopoietic progenitor cells derived from human embryonic stem cells (hESCs) or hiPSCs can be differentiated into sustained growth of erythroblasts (Hirose et al., ISSCR, Yokohama in 2012). This gene combination was determined by our hypothesis that an appropriate rage of c-MYC activation promotes erythroblast proliferation in the presence of erythropoietin (EPO), along with BCL-XL, an inhibiter of apoptosis enhanced by EPO stimulation. The self-replication of erythroblasts was regulated by a doxycycline (DOX)-dependent system.
Here we again attempted to make newer self-replicable erythroblasts, 5 individual clones from hESCs, KhES-3 (3 clone, Clone-8, Clone-16, Clone-A) or H1 (1 clone, Clone-B), or human dermal-fibroblast derived iPSCs (TkDN SeV2, 1 clone, Clone-C), all of which expressed CD235ab (Glycophorin A/B) and CD71 (transferrin receptor). Interestingly, we found individual erythroblast clones recapitulated different stages of development in erythropoiesis. For example, Clone-A showed proerythroblast-like morphology with faster doubling time, which resembled earlier phase in bone marrow erythroblasts. Next, as second example, Clone-8, Clone-16, or Clone-B displayed relatively mature phase, as exemplified by basophilic erythroblast-like morphology. As third sample, Clone-C showed most mature stage, as evidenced by polyerythroblast-like morphology with relatively slower self-replication capability. All 5 clones we established were capable of proliferating more than 3 months to date in the presence of overexpression of c-MYC and BCL-XL. After these genes were turned-off, mature signs including nuclear condensation, up-regula-

tion of GATA-1 or KLF1, or down-regulation of GCN5 were observed, indicating that DOX-system with defined two gene manipulation may contribute to the immortalization of erythroblasts enabling maturation.
In conclusion, we provide a new model for studying erythrocyte biogenesis towards clinical application while we have to further investigate whether original property of individual hESC/iPSC clones is associated with the different stages of self-replicable erythroblasts.

F-2151

APPLICATION OF IPS CELLS TO THE RESEARCH OF FIBRODYSPLASIA OSSIFICANS PROGRESSIVA

Yoshihisa Matsumoto[1], Makoto Ikeya[2], Edward Hsiao[3], Christopher R. Schlieve[3], Isao Asaka[2], Takanobu Otsuka[4], Bruce R. Conklin[5], Junya Toguchida[1]
[1]Institute for Frontier Medical Sciences, Kyoto Univ, Kyoto, Japan, [2]Center for iPS Cell Research and Application, Kyoto Univ, Kyoto, Japan, [3]Division of Endocrinology and Metabolism University of California- San Francisco, San Francisco, CA, United States, [4]Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan, [5]Gladstone Institute of Cardiovascular Disease, San Francisco, CA, United States

Fibrodysplasia Ossificans Progressiva (FOP) is a rare genetic disease characterized by progressive ectopic ossification, which severely inhibits patients' activities of daily living. The responsible gene for FOP is the ACVR1 gene, which is a type I receptor for BMP. Mutations found in patients transform ACVR1 protein into a constitutive active form, which transduces the BMP signal without a ligand binding. This causes ectopic ossification in muscles, tendons, and ligaments, although precise mechanisms are not yet known. Harvesting target tissues from patients is limited to special circumstances because tissue damage accelerates the ectopic ossification. This issue now can be overcome by using iPSCs derived from patients. We have established iPSCs from patients with FOP, from which cells in mesenchymal lineages are induced such as bone, cartilage, muscle, or tendon cells. We are currently analyzing the difference between wild type iPSCs and FOP-iPSCs during osteogenesis and chondrogenesis. We also try to recapitulate disease's phenotype in vitro by the stimulation with factors related to tissue damage. In this presentation, we will report our recent data and discuss the possibility of drug discovery by using our system.

F-2152

PATHOLOGICAL MODELING OF FRIEDREICH'S ATAXIA USING HUMAN INDUCED PLURIPOTENT STEM CELLS DERIVED NEURONS

Arielle Peche[1], Benjamin Foret[1], Aurélien Bayot[2], Cécile Denis[1], Laure Chatrousse[1], Mathilde Girard[1], Pierre Rustin[3], Marc Peschanski[1], **laetitia aubry**[1]
[1]INSERM/UEVE UMR 861, I-STEM, AFM, EVRY Cedex, France, [2]Inserm U676, Paris, France, [3]INSERM U676, Paris, France

Friedreich ataxia (FA) is an autosomal recessive neurodegenerative disease associated with spinocerebellar ataxia and hypertrophic cardiomyopathy. FA results from a GAA triplet repeat expansion within the first intron of the FXN gene encoding the mitochondrial protein frataxin. This mutation impairs transcription of frataxin leading to a significant reduction in mRNA and protein levels. To date explorations of FA molecular and cellular pathological mechanisms in different cellular and animal models suggests that oxidative stress plays a key and early role in the disease. Disruption of iron-sulfur cluster biosynthesis and iron homeostasis dysregulation have also been identified as pathological consequences of frataxin deficiency. However, despite the progress in understanding the pathophysiology of the disease, little is known about the neuronal defects resulting from FA mutation. Clearly, the development of efficient preventive or protective therapies is impeded by our limited knowledge of the neurobiology of these severe conditions. Thus, in spite of a wealth of existing and useful cellular models, the development of a new paradigm replicating the disease mains aspects more closely, i.e. the neurodegeneration and the cardiomyopathy, is of major interest. Human induced pluripotent stem cells (hiPS) represent a recent breakthrough in

stem cells and cellular reprogramming fields that offers new exciting perspectives in pathological modeling. hiPS cells can be obtained from patient and differentiate into cell types relevant for the pathology like neurons. Thus our project aims at generate hiPS-derived neural and neuronal cells carrying FA mutated gene to decipher the molecular mechanisms of the disease and identify new therapeutic compounds through large-scale drug screenings. To that purpose we first directly reprogrammed primary fibroblasts from three FA patients and three controls into hiPS cells lines. Characterization by immunocytochemistry and quantitative RT-PCR analysis showed a increase of pluripotency markers expression in hiPS cells lines compared with the parental fibroblast. Spontaneous differentiation into derivatives of the three embryonic germ layers was also confirmed *in vitro* through the formation of EBs. We then generated neural stem cells and neurons from control and FA-hiPS cells. Molecular analysis in neural cells and neurons revealed that these cells exhibited an increase expansion of GAA triplet repeats compare to their wild type counterparts. Moreover we showed a decrease expression in mRNA and protein level in FA neural cells and neurons. Altogether, these data indicate that FA hiPS cells and their derivatives represent a relevant cellular tool to investigate FA pathophysiology and identify novel FA biomarkers as well as new therapeutic compounds.

F-2153

TALENS™: PRECISE GENE EDITING OF HUMAN PLURIPOTENT STEM CELLS IN A ROBUST CULTURE SYSTEM, DEF-CS™

Catharina Ellerström[1], Tina Nilsson[1], Marianne Duhamel[2], Nathalie Ollivier[2], Dorra El Hajjam[1], Mikael Englund[1]
[1]Cellectis bioresearch, Göteborg, Sweden, [2]Cellectis bioresearch, Evry, France

Human pluripotent stem cells (hPS cells) provide unique possibilities
for efficient and relevant *in vitro* studies of human cells in basic research, disease modeling and in industrial applications. In some applications, genome customization is needed for repair of potential genetic-abnormalities. Similar approaches can be used in the generation of more mature differentiated cells and derivation of endogenous promoter driven reporter cell lines. By introducing relevant genome engineering technology, one will facilitate and improve safety pharmacology; toxicity testing and help scientists to better understand pathological processes in humans.
Genetic modification of hPS cells has been technically challenging. Many of the difficulties have been associated with suboptimal culture conditions and with poor survival of the hPS cells during the procedures. These difficulties made hPS cells less amenable to genetic manipulation. Today, robust culture protocols for culture of hPS cells are available and the efficiency of targeted engineering is greatly enhanced by the use of selectively cutting endonucleases, especially the recent TAL effectors nucleases (TALENs™).They can bind to any gene and introduce a specific DNA double strand break at any selected position. By combining a robust feeder free culture system, DEF -CS™, the TALEN™ technology allow insertion of a “Gene of Interest” into a defined locus. Further, to facilitate the analysis, the DEF-CS supports the hPS even through single cell sorting. This proved the user with an efficient, robust and precise approach to perform advance genome engineering in hPS.

F-2154

RESILIENCY OF H19 IMPRINTS IN EQUINE INDUCED PLURIPOTENT STEM CELLS

Mikhael Poirier, Jacinthe Therrien, Olivia Eilers Smith, France Filion, Lawrence Charles Smith
Centre de Recherche en Reproduction Animale (CRRA), Universite de Montreal, St-Hyacinthe, QC, Canada

Although the transformation of induced pluripotent stem (iPS) cells from somatic cells requires a process of epigenetic reprogramming, epigenetic marks controlling the expression of imprinted genes must remain untouched to retain their parental-specific expression patterns. H19, a paternally imprinted gene that plays an important role in development, is controlled through a differentially methylated region (DMR) containing CTCF binding sites. There have been reports in human iPS lines of extensive demethylation of the paternal allele indicating that reprogramming may affect genomic imprints. In order to confirm whether similar epigenetic modifications occur in equine

iPS cells, we analyzed the levels of methylation of the H19 DMR using a hybrid model, the mule. Single nuclear polymorphisms (SNP) were found in the H19 promoter sequence, permitting allele identification. Mule iPSc were obtained by transfecting foetal fibroblasts using a piggyBac (PB) transposon-based technique containing the reprogramming factors Oct4, Sox2, Klf4 and c-Myc and then morphologically selecting the transfected colonies. iPSc colonies were obtained from cloned fibroblasts of day 26 and day 42 fetuses. 4 iPSc colonies of each fibroblast source were obtained and analyzed. Bisulfite treatment (Epitect, Qiagen) permitted the observation of methylation patterns in subcloned sequences, that were then analyzed using BiQ Analyzer software. A region of 18 CpG island harboring the CTCF binding sites of the H19 DMR was analyzed. Complete methylation of the DMR was observed in paternal allele obtained in all colonies observed regardless of the age of fetus used for iPS generation, indicating that developmental age did not impact the resiliency of methylation of the paternal allele. As expected for H19 gene, maternal alleles revealed complete demethylation of the DMR. Therefore H19 genomic imprint maintains its insulator model through reprogramming into induced pluripotent stem cells. These results suggest that imprinted genes are resistant to epigenetic reprogramming required for producing equine iPS cells, thus permits the retention of proper parental marks in the DMR responsible for H19 activation.

F-2155

H3K27 DEMETHYLASE FUNCTION IN REPROGRAMMING PROCESS

Zhaoyu Lin, Anying Song, Xiang Gao
Model Animal Research Center of Nanjing University, Nanjing, China

Currently, the roadblock to clinical application of induced pluripotent stem cells (iPSCs) is safety. Understanding molecular mechanisms critical for reprogramming is one way to solve this problem. Histone methylation, especially H3K27 and H3K4 methylations, is important for epigenetic modifications, and plays key roles in reprogramming. During this process, H3K27 positions near pluripotent genes are demethylated and the ones near somatic genes are methylated. But how H3K27 demethylase affect reprogramming are still unknown. *Utx* and *Jmjd3*, which are the H3K27 demethylase genes, also have demethylation-independent functions. Though Utx null cells show impaired reprogramming capacity and Jmjd*3* knockdown cells seems normal, there are no direct evidence to exclude contributions of Jmjd3 or demethylation-independent functions of Utx. By generating and reprogramming H3K27 demethylase domain deletion somatic cell, we demonstrated these demethylases affected reprogramming. Thus, our results provide an opportunity to analyze the relationship between H3K27 methylation modifications and reprogramming.

F-2156

EFFICIENT DIFFERENTIATION OF DOPAMINERGIC (DA) NEURONS FROM CHEMICAL TREATED IPSCS

Hang-soo Park, Seung-Ick Oh, Insik Hwang, Han-Kyul Park, Sunghoi Hong
Lab. of Stem cell Biology, Department of Biomedical Science, seoul, Korea, Republic of

Induced pluripotent stem cells (iPSCs) represent a promising unlimited cell source for neurological diseases such as Parkinson's disease (PD) and Huntington's disease (HD). It has been reported that the treatment of various chemicals improved the efficiency of reprogramming during iPSC generation from somatic cells, and some of these chemicals were also used to increase neuronal differentiation. In this study, we report that mouse and human iPSC lines were established from fibroblasts using two different culture conditions, which is that one was treated with chemicals and the other was not treated during iPSC generation. Interestingly, we found that dopaminergic (DA) neurons were efficiently generated from chemical-treated iPSC lines compared to chemical-free iPSC lines using 5-stage and co-culture *in vitro* differentiation protocols. As a first step, we confirmed that the iPSC lines were positive against alkaline phosphatase (AP) and various ES markers such as Oct4, Nanog, Sox2 and SSEA-1, and were also differentiated into three germ layers *in vitro* and formed teratoma in SCID mice *in vivo*. Next, we directly compared the differentiation efficiency of DA neurons between chemical-treated and chemical-free iPSC lines. The efficiency of DA neurons that were differentiated from chemical-treated mouse iPSCs (miPSCs) was approximately two folds com-

pared to chemical-free miPSCs. The human iPSCs (hiPSCs) were induced into neural precursor (NP) cells and then were efficiently differentiated into TH^+ DA neurons. Interestingly, we found that the DA neurons out of βIII-tubulin$^+$ cells were approximately 75%, 8% and 34% from chemical-treated hiPSCs, chemical-free hiPSCs, and H1 human embryonic stem cells (hESCs), respectively. Therefore, these results showed that the iPSC lines that were treated with chemicals during reprogramming were efficiently differentiated into DA neurons, and also suggest that certain chemicals may be useful for iPSC generation and their differentiation into DA neurons for the cell-based therapy of PD.
Supported by the National Research Foundation (20120006453 and 2012050131) and the Ministry of Health and Welfare (A12039212010000300) Grants

F-2157

AUTOPHAGY MECHANISM IN REPROGRAMMING AND INDUCED PLURIPOTENT STEM CELLS

Sotthibundhu Areechun[1], Katya McDonagh[1], Agnieszka Klawiter[1], Kerry Thompson[2], Peter Dockery[2], Timothy O'Brien[1], Frank Barry[1], Sanbing Shen[1]
[1]Regenerative Medicine Institute (REMEDI), National University of Ireland, Galway, Ireland, [2]Department of Anatomy, National University of Ireland, Galway, Ireland

Autophagy is a normal cellular mechanism to maintain cell activity and viability in response to nutrient limitations and cell stresses, which can be altered under pathological conditions. There is also growing evidence that autophagy can modulate cellular reprogramming processes for the generation and differentiation of induced pluripotent stem (iPS) cells by chromatin remodeling and mitophagy. iPS cells have recently been used as a model system to study neurodevelopmental/psychiatric diseases. Understanding the links between autophagy and iPS generation may help to develop treatments for neurodevelopmental/psychiatric diseases. We have used a single lentiviral vector and established human iPS cell lines from healthy donors and patients with deletions/mutations on *NRXN1* and *FMR1* genes. We examined the baseline activity of autophagy in iPS cells from healthy donors and found expression of various autophagy components (including LC-3B, Beclin-1, Atg3, and Atg12) in different iPS cell lines. Currently we are investigating whether autophagy proteins are differentially expressed in healthy and disease iPS cells at the subcellular and molecular levels, and whether manipulation of the autophagy activity influences iPS cell maintenance and differentiation.

F-2158

ESTABLISHMENT OF AN INDUCED PLURIPOTENT STEM CELL BIOBANK AT CORIELL INSTITUTE FOR MEDICAL RESEARCH

Karen Fecenko-Tacka, **Shilpa Gandre-Babbe**, Ken George, Steven Madore, Dorit Berlin
Coriell Institute for Medical Research, Camden, NJ, United States

Induced pluripotent stem cells (iPSCs) represent a powerful new tool for studying human disease at the cellular level. These cells, first established using viral-mediated reprogramming of fibroblasts, have also been derived from other cell types. More recently, reprogramming has been accomplished using episomal vectors, mRNA, or small molecules. A significant number of the first iPSC lines were derived from fibroblasts originating from the the NIGMS Human Genetic Cell Repository and the NIA Cell Repository collections at Coriell. Many of these iPSCs were processed by the Coriell Stem Cell Biobank [SCB]. The goal of the SCB is to generate high quality iPSCs submitted by the research community or made at Coriell using standard reprogramming methodology. A large number of iPSC lines are available for research purposes through the Coriell Cell Repositories web catalog (ccr.coriell.org).

Biobanking of iPSCs requires a systematic workflow with extensive molecular and cellular characterization and quality control testing. iPSCs expanded for distribution must undergo viability testing, microbial contamination testing (including PCR-based mycoplasma screening) and DNA fingerprint analysis. In addition, chromosomal integrity is assessed using G-banded karyotype analysis and genome-wide SNP genotyping. Surface expression of pluripotency markers is determined via flow cytometry, and each distribution lot is tested for embryoid body formation and directed differentiation toward lineages representative of the three germ layers. A panel of qPCR assays has been assembled to efficiently screen steady-state mRNA expression of the cells in the undifferentiated state, after embryoid body formation and after directed differentiation. For some iPSC lines, teratoma formation assays were performed to assess *in vivo* pluripotency. Cell lines are made available for research purposes to academic, non-profit and industry investigators after completion of a material transfer agreement. Customers will be supplied with a Certificate of Analysis for each cell line, culturing protocols and troubleshooting tips. These processes developed and implemented by Coriell Cell Repositories will aid in establishing best practices for iPSC biobanking and distribution. We present our processes for iPSC banking along with representative data from lines available through the NIGMS Human Genetic Cell Repository.

F-2161

GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Methichit Wattanapanitch[1], Tanapol Phondeecharoen[2], Yaowalak U-pratya[3], Chuti Laowtammathron[1], Nuttha Klincumhom[1], Surapol Issaragrisil[3]

[A]Siriraj Center of Excellence for Stem Cell Research, Faculty of Medicine Siriraj Hospital, [B]Department of Immunology, Faculty of Medicine Siriraj Hospital, [C]Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, [1]Mahidol University, Bangkok, Thailand

Paroxysmal nocturnal hemoglobinuria (PNH) is a non-malignant clonal disorder characterized by chronic hemolytic anemia, hemoglobinuria, cytopenia and high incidence of thrombosis. A major cause of the disease is due to somatic mutations of the *Phosphatidylinositol glycan A* (*PIG-A*) gene in hematopoietic stem cells (HSCs), which result in CD55 and CD59 deficiency on blood cell membrane, leading to complement-mediated cell lysis. At present, treatment for patients with PNH aims at preventing the adverse complications; however, patients still require lifelong monitoring of clinical manifestations and medications. Hematopoietic stem cell transplantation (HSCT) offers a possible cure for the disease. Nonetheless, allogeneic HSCT is limited by lack of human leukocyte antigen (HLA)-matched sibling donors and the likelihood of developing graft versus host disease (GvHD). Human embryonic stem cell (hESC)-derived HSCs are a potential source of hematopoietic stem cells for allogeneic HSCT but ethical problems and different HLA typing are still major concerns. Induced pluripotent stem cell (iPSC)-derived HSCs are considered to be a potential source of cells for autologous HSCT. Here we report the generation of human iPSCs from PNH patients' skin fibroblasts (PNH-iPSCs) by the retroviral transduction of four reprogramming factors: OCT4, SOX2, KLF-4 and c-MYC. The PNH-iPSCs expressed pluripotency-associated markers as examined by using immunofluorescent staining and real-time PCR analyses. Furthermore, PNH-iPSCs could spontaneously differentiate into cells of three embryonic germ layers via embryoid body formation. Interestingly, our study indicated that the PNH-iPSCs expressed CD59 at similar levels to those of the normal human dermal fibroblast-derived iPSCs. Currently, karyotypic analysis and hematopoietic differentiation are being investigated to validate that hematopoietic stem/progenitor cells derived from PNH-iPSCs are normal, i.e. express CD59 at similar levels to those of normal hema-

topoietic stem/progenitor cells. The finding from this study may provide a promising autologous source of HSCs for transplant in PNH patients.

F-2162

STUDYING THE ROLE OF BMYB IN SOMATIC CELL REPROGRAMMING

Carl Ward[1], Cesar A. Sommer[2], George Murphy[2], Keisuke Kaji[3], Jon Frampton[1], Paloma García[1]
[1]Immunity and Infection, University of Birmingham, Birmingham, United Kingdom, [2]Department of Medicine, Boston University, Boston, MA, United States, [3]School of Biological Sciences, Institute for Stem Cell Research, Edinburgh, United Kingdom

Mice generated without Sox2 or Oct4 fail to develop past the blastocyst stage due to impaired formation of the inner cell mass. This phenotype is mirrored in mice lacking the transcription factor B-Myb, highlighting the importance of B-Myb during the early stages of embryonic development. In fact, B-Myb transcripts are 1000-10000 times more abundant in embryonic stem cells (ESCs) compared to other somatic cells. Our previous work demonstrated that B-Myb does not affect the expression of Oct-4 and Sox-2 levels, and thus is unlikely to play any role in the maintenance of pluripotency. However, B-Myb controls replication dynamics necessary to maintain genome stability, possibly through the action of key multifunctional proteins such as c-Myc.
In cellular reprogramming, induced pluripotent stem cells (iPSCs) are successfully generated upon exogenous expression of a combination of four factors, namely Oct-4, Sox-2, Klf-4 and c-Myc. Among these, c-Myc can be spared from the cocktail of factors and the endpoint of reprogramming still achieved, although efficiency is significantly reduced and the timing of iPSC appearance is increased considerably.
Our hypothesis is that the increase in reprogramming efficiency obtained in the presence of c-Myc is related to the unique cell cycle properties of ESCs, and that B-Myb is likely to play an important role in the maintenance of genome fidelity and acquisition of pluripotency during reprogramming through the regulation of the c-*myc* gene.
In agreement with a role of B-Myb during reprogramming, Keisuke and colleagues have shown that B-Myb RNA levels start increasing at day 5, earlier than other pluripotent factors such as nanog.
In order to evaluate the importance of B-Myb during reprogramming we would like to answer the following questions: (i) Can B-Myb substitute c-myc in the reprogramming cocktail without efficiency being affected? (ii) Can the addition of B-Myb increase the efficiency of reprogramming in the presence of the four reprogramming factors? (iii) Can cells lacking B-Myb reprogram? If not, in which step during reprogramming is B-Myb necessary?
To answer these questions we have firstly tried to generate of iPSCs using Oct-4, Sox-2, Klf-4 and c-Myc in the absence of B-Myb. By using a polycistronic lentiviral vector encoding the transcription factors Oct4, Sox2, Klf4 and c-myc we have preliminary results showing the failure of somatic reprogramming of *B-Myb* $^{\Delta/\Delta}$ MEFs.
Secondly, we have tried the generation of iPSCs using Oct-4, Sox-2, Klf-4, c-myc and B-Myb. Surprisingly, when B-Myb is overexpressed at the time of reprogramming the efficiency of reprogramming was significantly lowered when analysed by counting the number of positive alkaline phosphatase colonies.
These results show that B-Myb has an important role in reprogramming and should be investigated further.

F-2163

DEVELOPMENT OF NOVEL TECHNOLOGIES FOR THE GENERATION OF CGMP-COMPLIANT INDUCED PLURIPOTENT STEM CELLS

Thomas Fellner, Xinghui Tian, Inbar Friedrich Ben-Nun, Ying Nie, Patrick Walsh, Jean Cadet, Amy Burkall, Huan Tran, Kevan Shah, Behnam Ahmadian Baghbaderani
LONZA, Walkersville, MD, United States

In 2007, Dr. Shinya Yamanaka became the first to successfully convert adult human cells to induced pluripotent stem cells (iPSCs). These cells have similar characteristics to embryonic stem cells (ESCs) including the potential to become any cell type in the body. It is therefore thought that human iPSCs (hiPSCs) can be utilized in cell therapies

for the treatment of a multitude of diseases. While human ESCs are limited to allogeneic therapies, hiPSCs can be used for the development of both allogeneic and autologous therapies.
The production of clinical-grade hiPSCs is regarded as a critical milestone necessary to realize the therapeutic potential of these cells. However, to reach this milestone, numerous challenges need to be overcome; the first of which is production of clinical-grade hiPSCs under current Good Manufacturing Practices (cGMP). As an important first step to achieving this goal, we have developed a defined, cGMP-compliant cell culture system consisting of a medium, matrix and passaging method. Utilizing this culture system in combination with a novel miRNA-enhanced "zero-footprint" reprogramming technology, we have demonstrated efficient generation of hiPSCs from blood cells. The resulting hiPSCs share characteristics with human ESCs, including the expression of ESC-associated markers. In addition, these hiPSCs can efficiently differentiate into cells of all three germ layers and have a normal karyotype. Importantly, hiPSCs show no trace of exogenous DNA integration, confirming that cells were reprogrammed with a "zero-footprint" technology. At this conference we intend to present the experimental data used to design a cGMP compliant reprogramming and culture system.

F-2164

MODELING ALZHEIMER'S DISEASE WITH PATIENT SPECIFIC IPS CELLS**<!--EndFragment-->**

Mansoureh Shahsavani[1], **Mari Pekkanen-Mattila**[2], Anna Falk[1], Anna Herland[2]
[A]Department of Neuroscience, [B]Department of Cell and Molecular Biology, [1]Karolinska Institute, Stockholm, Sweden

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases and the cause of dementia. Dementia and AD are becoming more prevalent with an aging population, but also younger people are affected. Today, about 1 person in every 1000 below the age of 65 develops dementia. A vast majority (> 95 %) of the AD cases are spontaneous, but there are also inheritable, familial, variants of the disease. Apart from neurodegeneration, two major hallmarks of AD are extracellular deposits of Aβ peptides, so called amyloid plaques, and intracellular accumulation of filamentous hyperphosphorylated Tau protein, neurofibrillary tangles.
Our aim is to model Alzheimer's disease with patient specific iPS cell derived neuronal cells. iPS cells from an AD patient with London mutation (APP717) as well as from a healthy person matched with age and an ApoE genotype are to be generated. iPS cells production is made by using non-integrating viral vectors and they are characterized as a pluripotent stem cells by their gene and protein expression profile, in addition to their differentiation potential.
The neuronal differentiation of the AD derived iPS cells is to be performed via the neuroepithelial stem (NES) cell stage. NES cells can be robustly expanded in vitro without losing their potential to differentiate into neurons and glia. For AD disease modeling a protocol for directing iPS cell derived NES cells towards forebrain cholinergic neurons is to be developed. In addition to neuronal characterization, these differentiated cells are to be evaluated with AD specific assays e.g. ratio of secreted amyloid beta (Aβ) peptides and phosphorylation of Tau.
The disease specific and patient specific iPS cell lines were generated. Furthermore, these iPS cell lines were used to generate NES lines, which were stable in long-term culture and differentiated into forebrain cholinergic neurons. The results from analyzing the patient and control cholinergic neurons suggest that both have increased production of Aβ during neuronal maturation. However, results reveal the increased Aβ 42/40 ratio in the patient cells when compared to control. In addition, a difference between phosphorylated Tau protein level in AD patient and WT cells was seen. Morphology wise it can be concluded that in the AD patient the phosphorylated Tau is localizing in the neurites showing a disease phenotype in vitro.
As a conclusion, the data indicates that it is possible to model the neurodegenerative disease in vitro by using iPS cell technology. Furthermore, these models can be used in studying the pathophysiology and mechanisms of neurodegenerative diseases in the future.

F-2165

DIRECT DIFFERENTIATION OF PATIENT IPS CELLS INTO SELF-RENEWING NEURAL PROGENITORS BY SMALL MOLECULES TO MODEL MITOCHONDRIAL DISEASES

Raul Bukowiecki[1], Vanessa Pfiffer[1], Sheila Hoffmann[1], James Adjaye[2], Erich E. Wanker[1], Alessandro Prigione[1]
[1]Neuroproteomics, MDC Berlin, Berlin, Germany, [2]Regenerative Medicine, Heinrich-Heine-Universitaet, Duesseldorf, Germany

Here, we report a rapid and feasible method to derive self-renewing neural progenitor cells (NPCs) from human pluripotent stem cells (PSCs). With an approach adapted from Li et al. (PNAS, 2011), similar results could be obtained for human embryonic stem cells (ESCs) and human induced pluripotent stem cells (iPSCs) generated from fibroblasts of three patients and control individuals. All patients carry a mitochondrial DNA (mtDNA) mutation in MT-ATP6. Mutations in this gene -encoding a mitochondrial ATPase subunit- are associated with neurodegenerative disorders ranging from adult-onset NARP (Neurogenic weakness, Ataxia, and Retinitis Pigmentosa) to severe infantile Leigh syndrome (LS). Importantly, we were able to generate multiple iPSC clones (patient and control) employing episomal plasmid-based reprogramming and thus avoiding traditional viral-mediated transgene delivery. All patient iPSCs still displayed the same mutational load in mtDNA as their parental fibroblasts, even upon prolonged cultivation.
Our protocol exhibits major advantages in comparison to standard methods of obtaining NPCs. First, it does not require the formation of embryoid bodies (EBs). Second, it is operator-independent, as it bypasses the need for tedious manual isolation of neural rosettes. A combination of human leukaemia inhibitory factor (hLIF), a GSK3ß inhibitor (CHIR99021), and a TGFß inhibitor (SB431542) in chemically defined media was sufficient to induce the conversion of iPS cells to highly proliferating SOX2 and NESTIN-positive NPCs (98 %). The obtained NPC monolayer population shows distinct morphological changes within a very short time (7-10 days) and could be cultured over several passages without loss of proliferation. Moreover, NPCs derived from various patient iPSC lines underwent in-depth characterization and were capable of differentiating into neural as well as glial subtypes, which was confirmed by expression of markers like TUJ1, HB9, ISL1, GFAP, and CNP.
Additionally, patient-derived NPCs were subjected to metabolic analysis, e.g. ATP quantification and Seahorse-based bioenergetic profiling. In these assessments, we could observe responsiveness of NPCs to dihydrolipoic acid (DHLA), an oxygen radical scavenger currently being tested for the treatment of NARP patients (Couplan, PNAS, 2011). DHLA was capable to improve respiratory activity in patient NPCs to nearly 2-fold and to restore mitochondrial functionality upon conditions mimicking glucose shortage, which represents a potential cause of decompensation in NARP/LS patients. Thereby, a novel method of determining the metabolic state of neural tissue could be established, potentially enabling the study of mitochondrial encephalopathies and unravel their underlying molecular mechanisms.
Overall, NPCs from patient iPSCs represent an inexhaustible source of neurogenic tissue. We previously found that retroviral-mediated reprogramming may result into mtDNA sequence rearrangement (Prigione et al, Stem Cells 2011). Hence, non-viral generation of iPSCs, followed by small molecule-based derivation of NPCs may represent an advantageous strategy to establish faithful neuronal disease models with positional effect-free phenotypes, thus guarantying reproducibility and genomic stability.

F-2166

GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS FROM NON-CRYOPRESERVED BANKED BRAIN TISSUE

Lauren B. Vensand[1], Andrew A. Sproul[1], Carmen R. Dusenberry[1], Jean Paul G. Vonsattel[2], Michael Shelanski[2], John F. Crary[2], Scott A. Noggle[1]
[1]The New York Stem Cell Foundation, New York, NY, United States, [2]Pathology & Cell Biology, Columbia University, New York, NY, United States

Donated brain tissue collected in public biobanks has the potential to provide a tremendous resource as starting material for the generation of disease-specific induced pluripotent stem cells (iPSCs). For instance, post-mortem

analysis of brain tissue can allow definitive classification of classical Alzheimer's disease versus other forms of dementia, which is still challenging to assess in live patients .The majority of banked material is not stored with intention of growing functional tissue, and thus is frozen without the addition of cryoprotectants such as DMSO. As a pilot experiment, we successfully generated iPSCs from cryoprotected frozen dural and scalp tissues from an individual who had Multiple Systems Atrophy, a fatal neurodegenerative disease. Subsequently, we were able to get outgrowth from non-cryoprotected dural tissue from four of nine classical Alzheimer or control individuals. We were able to successfully reprogram dural cells from one Alzheimer's brain that had been in frozen storage for nine years. Thus, one can generate iPSC lines from valuable autopsied banked tissue including very rare patient material.

F-2167

GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM PRIMARY HUMAN B LYMPHOCYTES

Makoto Inaki, Masafumi Onodera
Department of Genetics, National Research Institute for Child Health and Development, Tokyo, Japan

Induced
pluripotent stem (iPS) cells are an attractive tool for not only comprehension of the mechanisms underlying various diseases but also applications in regenerative medicine. Recent improved technologies have allowed us to generate human iPS cells from various somatic tissues including hematopoietic cells. In particular, peripheral blood cells seem to be ideal because a relatively large number of cells can be obtained at a time with little invasiveness. To date, such iPS cells have been generated from T lymphocytes or $CD34^+$ cells derived from peripheral blood. However, to the extent of our knowledge, the establishment of iPS cells from peripheral B lymphocytes has not been reported except for those derived from Epstein-Bar virus transformed lymphoblastoid B-cell lines (EBV-LCLs). Here we have presented the method to generate iPS cells from human peripheral B lymphocytes (BiPS). Human $CD19^+$ cells isolated from peripheral blood of healthy individuals were activated by cytokines (IL-2, IL-4 and IL-21) together with the concentrated culture supernatant of a CD40L-expressing cell line, and then transduced with genes encoding for the Yamanaka factors (OCT4, SOX2, KLF4, and cMYC) and enhanced green fluorescent protein (EGFP) using retroviral vectors (PG13/OSKMG) followed by culture on Mitomycin-C treated mouse embryonic fibroblasts in the presence of basic FGF. Accordingly, cells that were morphologically similar to human embryonic stem cells appeared at three weeks of culture. They were stained with alkaline phosphatase and markers for pluripotency and capable of forming teratomas containing tissues derived from all three germ layers when transplanted in immune incompetent mice. The complementarity determining region 3 (CDR3) of their immunoglobulin heavy chain (IgH) gene was hyper-mutated compared with that of non-B cell lineages, suggested that they were derived from mature B lymphocytes. BiPS may provide a useful tool for analysis of diseases with a deficit of peripheral T lymphocytes such as X-linked Severe Combined Immunodeficiency (SCID-X1).

F-2168

ERYTHROPOIETIC POTENTIAL OF IPS CELLS DERIVED FROM SHWACHMAN-DIAMOND SYNDROME

Christine M. Miller, Thomas F. Serwold, Amy J. Wagers
Developmental and Stem Cell Biology, Joslin Diabetes Center, Boston, MA, United States

Null mutations in the Shwachman-Bodian-Diamond Syndrome (SBDS) gene cause Shwachman-Diamond syndrome (SDS), a rare ribosomopathy that affects the bone marrow, pancreas and skeletal system. Patients with SDS exhibit a predisposition for Acute Myeloid Leukemia (AML) as well as defects in erythropoiesis. Although the exact mechanism underlying defective erythropoiesis in these patients is unknown, previous studies suggest that erythrocyte formation from differentiated hematopoietic cells is not impaired; instead, erythrocytes of SDS patients appear to undergo Fas-mediated apoptosis at an elevated rate. In this study, we utilized induced pluripotent stem (iPS) cells from SDS patients and subjected them to erythroid differentiation protocols in order to obtain a more complete developmental view of SDS erythropoiesis. To stimulate hematopoietic and erythroid differentiation, we co-cultured iPS cells with a mono-layer of gene-modified mouse OP9 cells using differentiation media supplemented with hematopoietic cytokines. Flow cytometric analysis demonstrated the emergence of CD29-, CD31+/CD43+, CD235a+/CD71+ human cells, suggesting erythroid differentiation, and Wright-Giemsa staining of FACS-sorted cells confirmed the erythroid phenotype. We also evaluated the hematopoietic progenitor potential of these cells using in vitro colony-forming assay. These studies establish a useful method to recapitulate the complete erythropoietic differentiation pathway in SDS patients by exploiting the pluripotency of human iPS cells.

F-2171

SKELETAL MUSCLE DIFFERENTIATION OF HUMAN IPS CELLS AND GENERATION OF INSULIN RESISTANCE DISEASE MODEL

Salvatore Iovino[1], Cong Xu[2], Leonard I. Zon[2], Amy J. Wagers[3], Ronald C. Kahn[1]

[1]Integrative physiology and metabolism, Joslin Diabetes Center, Harvard Medical School, Boston, MA, United States, [2]Stem Cell Program and Hematology/Oncology, Children's Hospital and Dana-Farber Cancer Institute, HHMI, Harvard Stem Cell Institute, Harvard Medical School, Boston, MA, United States, [3]Stem Cell and Regenerative Biology, Joslin Diabetes Center, HHMI, Harvard Stem Cell Institute, Harvard University, Boston, MA, United States

Human induced-pluripotent stem cells (hiPSC) represent a potentially unlimited source of patient-specific differentiated cells, including myogenic cells, that could be used for the treatment and understanding of muscle and metabolic diseases. Current protocols to achieve skeletal muscle differentiation from hiPSC rely mainly on genetic manipulation which may have consequences for cellular physiology and therapeutic utility. We sought to define a novel protocol to differentiate hiPSC into skeletal muscle using small molecules and use this to study individuals with genetic insulin resistance. To this end, we performed a high-throughput image-based screen using transgenic Zebrafish blastomeres carrying myf5-GFP to label muscle progenitors and mylz2-mCherry to label differentiated myotubes. We found that forskolin (cAMP activator), 6-bromoindirubin-3'-oxime or Bio (GSK inhibitor) and FGF2 promoted myogenesis from blastomeres. To determine if these factors would also stimulate hiPSC differentiation, we treated embryoid bodies (EB) derived from hiPSC lines with a combination of these agents and analyzed skeletal muscle differentiation by RT-PCR and immunocytochemistry. We found that Pax7, Myf5, MyoD1 were selectively up-regulated during EB differentiation and after 6 weeks of treatment, hiPSC underwent terminal differentiation to mature myofibers developing multinucleated cells expressing myogenin and myosin heavy chain. These myofibers also exhibited skeletal muscle ultra-structures by electron microscopy and immunofluorescent staining for desmin. In addition, hiPSC harvested from forskolin/Bio/FGF2-treated cultures showed myogenic engraftment in an immunodeficient mouse which received cardiotoxin to induce muscle injury. Using this system we generated a model of insulin resistance using hiPS-derived skeletal muscle from 3 healthy subjects and 3 subjects affected by Donohue syndrome, a syndrome characterized by INSR (insulin receptor) mutation and severe insulin resistance. hiPS-derived skeletal muscle of Donohue syndrome showed reduced insulin signaling and impaired insulin-stimulated glucose uptake, recapitulating the features of the human disease. In conclusion, a combination of small molecules can drive hiPSC differentiation toward the skeletal muscle lineage and can be used to generate a disease model of insulin resistance.

F-2172

EPIGENETIC BARRIERS TO X CHROMOSOME REACTIVATION DURING REPROGRAMMING TO INDUCED PLURIPOTENCY.

Anu Sadhu Dimashkie
Biological Chemistry, UCLA-CSUN CIRM Bridges Student, Los Angeles, CA, United States

The differentiated state of somatic cells can be reprogrammed to induced pluripotent stem cells (iPSCs). In female somatic cells, the inactive X chromosome (Xi) is very stable however, upon reprogramming of mouse embryonic fibroblasts, the Xi reactivates. How the somatic character is reversed as well as the sequence of epigenetic events leading to X chromosome reactivation during reprogramming to iPSCs are not well understood. We found that, the non-coding RNA Xist and DNA methylation are present on the Xi in clonal late reprogramming intermediates (pre-iPSCs), suggesting that these are reversed only late during reprogramming. To test whether these function in maintaining Xi repression, we interfered with Xist and DNA methylation in pre-iPSCs using genetic ablation for Xist and/or treatment with siDNMT1 (DNA methyltransferase 1) and the DNA methyltransferase inhibitor 5AzadC (5-aza-2'-deoxycytidine). We found that reactivation of the Xi occurs upon deletion of Xist and inhibition of Dnmt1, but not when either pathway is lost alone. We used bisulfite sequencing to confirm the loss of DNA methylation state of X-linked genes in pre-iPSCs upon inhibition of Dnmt1. These data suggest DNA demethylation is required to allow for the reactivation of the Xi and that Xi-reactivation occurs very late in reprogramming. To further test this, we purified SSEA1+ population directly from a reprogramming culture. The SSEA1+ cell population is strongly enriched for cells that are near to the completion of reprogramming and represent a state where pluripotent marker Nanog is already active but the Xi still silent. In the SSEA1+ intermediate, we find that the promoter region of Nanog is already demethylated. However, promoter regions of Xi-linked genes are still completely methylated. Together, our results identify new late stages of reprogramming to iPSCs and demonstrate that DNA demethylation is a step-wise process.

F-2173

DETAILED ANALYSIS OF THE GENETIC AND EPIGENETIC LANDSCAPE OF IPS CELL-DERIVED MESODIENCEPHALIC DOPAMINERGIC NEURONS

Reinhard Roessler[1], Sebastien Smallwood[2], Jesse Veenvliet[3], Koushik Chakrabarty[3], Jeroen Pasterkamp[4], Marian Groot-Koerkamp[5], Gavin Kelsey[2], Erik Boddeke[6], Marten Smidt[3], **Sjef Copray**[6]
[1]Whitehead Institute for Biomedical Research, Cambridge, MA, United States, [2]The Babraham Institute, Cambridge, United Kingdom, [3]Centre for Neuroscience, Swammerdam Institute for Life Science, Amsterdam, Netherlands, [4]Dept. Neuroscience, Rudolf Magnus Institute of Neuroscience, Utrecht, Netherlands, [5]Molecular Cancer Research, University Medical Center Utrecht, Utrecht, Netherlands, [6]Dept. Neuroscience, Univ Medical Centre Groningen, Groningen, Netherlands

Induced pluripotent stem cells (iPSCs) have significant developmental potential and harbor great promise for *in vitro* generation of disease-relevant cell types, such as mesodiencephalic dopaminergic (mdDA) neurons, the neuron type centrally involved in Parkinson's disease (PD). IPSC-derived midbrain DA neurons have been generated, but detailed genetic and epigenetic characterization of strictly purified *in vitro* generated mdDA neurons is still lacking. Such in-depth knowledge is required before the next steps towards disease modeling and cell-based therapy for PD can be taken.
In order to elucidate genetic and epigenetic features of *in vitro* generated mdDA neurons, we selectively FACS-purified mdDA (Pitx3$^{gfp/+}$) neurons derived from mouse iPSCs and mdDA neurons newly formed during mouse development. Pitx3 is a highly specific mesodiencephalic dopaminergic (mdDA) neuron marker, which is required for DA neuron differentiation in the substantia nigra. We performed global comparative gene expression analysis with iPSC-derived Pitx3-GFP-positive cells and primary isolated mdDA neurons of several developmental stages (E12.5 till P0). Sample cluster analysis revealed strongest gene expression correlation of iPSC-derived mdDA neurons with embryonic primary neurons, whereas postnatal stage primary mdDA neurons clustered separately.

Next, comprehensive DNA methylation profiling by Reduced Representation Bisulfite Sequencing (RRBS) was performed. We obtained information on 844,812 CpGs (≥5 read depth) for iPSC-DA neurons and mdDA neurons, with bisulfite conversion efficiency higher than 98% as assessed by non-CpG methylation. Of these, 72% correspond to CGIs in iPSC-DA neurons and mdDA neurons. Global methylation levels outside of CGIs are similar in iPSC-DA and mdDA neurons, and are comparable to somatic tissues in general. Interestingly, the general methylation pattern seemed to be comparable between iPSC-derived DA neurons and primary mdDA neurons, indicating that our *in vitro* generated DA neurons widely adopt the epigenetic signature of their primary counterparts. Nonetheless, we also found deviations in CGI methylation for a subpopulation of 2000 genes, which effected predominately expression of genes involved in neuronal functions.
So, although IPSC-derived DA neurons do adopt most characteristics of their *in vivo* counterparts, as can be seen in morphology, dopamine production, global gene expression and CpG island (CGI) methylation profiles, abnormalities are still present. Such abnormalities need to be addressed and corrected prior to future translational approaches, since they might affect proper functionality of iPCS-derived mdDA neurons. Our findings underline the importance of high-throughput genome-wide screens of iPSC-derived cell types to evaluate their true potential for *in vitr*o disease modeling or cell-based therapy.

F-2174

CELL IDENTITY TRANSITIONS DURING INDUCED REPROGRAMMING TO PLURIPOTENCY

Shin-Il Kim, Fabian Oceguera-Yanez, Toshiko Sato, Kotaro Ohnishi, Takuya Yamamoto, Yasuhiro Yamada, Shinya Yamanaka, Knut Woltjen
Reprogramming Science, CiRA, Kyoto University, Kyoto, Japan

Reprogramming of somatic cells to induced pluripotent stem cells (iPSCs) by transcription factor overexpression represents a paradigm where exogenous transcriptional activator/repressor proteins are implemented to elicit changes in gene expression, ultimately altering cell fates. Oct3/4, Sox2, cMyc, and Klf4 (the “Yamanaka factors”) are capable of re-wiring a somatic cell to achieve pluripotency.
We have developed doxycycline (dox)-inducible, combinatorial transgene systems for reprogramming mouse somatic cells, permitting observation of dynamic changes in response to fixed factor stoichiometries. We employ these composite systems in MEFs to reveal direct effects mediated by reprogramming factor levels throughout the process. We uncovered a fundamental cause of distinct protein expression levels, which reproducibly manifests disparate effects on proliferation, morphology and colony formation, through differential modulation of the severity of the mesenchymal-epithelial transition (MET). How these differences initiate and maintain throughout the process, and their relevance to the final stages of reprogramming form the focus of our study. We find that subsequent late-stage factor expression levels impact endogenous pluripotency gene expression and apparent iPSC quality. Our data implies discrete reprogramming factor requirements at each stage of the process, and prospective refinement of current reprogramming standards.

F-2175

MODELING INSULIN RESISTANCE THROUGH iPSC TECHNOLOGY

Ivan Carcamo-Orive[1], Paige Cundiff[3], Sunita D’Souza[3], Hope Lancero[2], Caroline Hendry[3], Ana Sevilla[3], Fahim Abbasi[2], Gerald M. Reaven[2], Eric Schadt[4], Ihor Lemischka[3], Joshua W. Knowles[1], Thomas Quertermous[2]
[A]Cardiovascular Institute, [B]Cardiovascular Medicine, [1]Stanford School of Medicine, Stanford, CA, United States, [A]Developmental and Regenerative Biology, [B]Institute for Genomics and Multiscale Biology, [2]Mount Sinai School of Medicine, New York, NY, United States

Insulin resistance (IR) is a condition with serious health consequences due to its relationship with type 2 diabetes and cardiovascular disease. It is estimated that 25-33% of the US population has the IR syndrome and IR is expected to increase in prevalence as the world becomes heavier and more sedentary. Although much is known about the

physiological changes occurring during IR, little is known about the molecular pathways that drive the appearance of IR and the associated clinical syndromes. Certain mature cell types such as adipocytes, endothelial cells and skeletal muscle cells have been associated with the origin, maintenance and progression of IR. The limited accessibility to primary cells and the absence of genome-wide data for human samples have limited so far our knowledge of IR in humans. The emergence of induced pluripotent stem cells (iPSCs) as a powerful tool in the field of regenerative medicine offers an unprecedented opportunity of modeling human disease *in vitro*. The present study aims to take advantage of the genome-wide genotyping data previously obtained from insulin resistant (*IR*) *vs.* insulin sensitive (*IS*) patient groups to establish a cohort of iPSC lines. Differentiation of these iPSCs to relevant metabolic and vascular cell types will provide an unparalleled opportunity to correlate human insulin sensitivity and high-density genetic variation data with specific cell-based profiling, paving the way for a novel approach to characterize the cellular and molecular basis of insulin resistance. In a preliminary study we have begun to validate the approach as a disease model system. We have successfully generated several iPSC lines from different individuals with varying degrees of insulin sensitivity as measured by the insulin suppression test. Moreover, iPSC-derived mesodermal progenitor cells (iMPC) have been differentiated to the osteogenic and adipogenic lineages. Although iMPCs share a common surface marker profile, morphology and osteogenic lineage potential with canonical adult MPCs, the adipogenic differentiation can only be achieved by means of PPAR2 overexpression. In addition, we have generated and purified $CD31^+$ endothelial cells from iPSCs (iENDO). iENDO display similar characteristics of adult endothelial cells including surface markers by FACS (CD144, MCAM, endoglin), vascular tubule formation and acetylated-LDL uptake. Importantly, we have observed a differential insulin response (measured by Phospo-AKT levels) as well as differences in growth rate and expansion potential between *IR vs. IS* iENDO cells. Finally, iENDO cells from insulin resistant individuals show an increased response (V-CAM protein levels) when exposed to pro-inflammatory signals (TNF-α), indicating potential endothelial cell dysfunction. Our results in the endothelial lineage suggest that iPSC technology could allow, at least to some extent, the modeling of a multifactorial disease like insulin resistance.

F-2176

ROLES OF DNA REPAIR DEFICIENCY IN AGING ASSOCIATED DECLINE IN PLURIPOTENCY INDUCTION

Percy L. Yeung[1], Yasunari Seita[1], Dai-Tze Wu[2], Zhiyuan Shen[3], Monica Roth[2], Chi-Wei Lu[1]

[1]Child Health Institute, UMDNJ-RWJMS, New Brunswick, NJ, United States, [2]Department of Pharmacology, UMDNJ-RWJMS, Piscataway, NJ, United States, [3]CINJ, UMDNJ-RWJMS, New Brunswick, NJ, United States

Induced pluripotent stem cells (iPSCs) represent reprogramming differentiated somatic cells into a proliferative multipotent state, providing a hopeful autologus tissue source for regenerative medicine. Reprogramming aging tissues is of particular interest for its application in tissue regeneration, however, several studies have implicated complex molecular mechanisms in reprogramming aging tissues. In this study, we investigated the mechanism of aging effects on iPS reprogramming efficiency in the human. From a collection of 8 skin-biopsy derived primary fibroblasts, reprogramming efficiency varies with age while extreme low efficiency is commonly observed in several samples with advanced ages (more than 70 years old). Cell proliferation activities and p53 expression levels in these fibroblasts were evaluated and found no correlation between the age of the subject that fibroblasts were isolated. Interestingly, through monitoring p53 activities in response to DNA damaging γ-irradiation, sustained p53 & p21 levels was observed indicating the incompletion of DNA damage repair response. We found that in fibroblasts with poor reprogramming efficiency, DNA double strand break repair activities reflected by RAD51 foci formation after irradiation is significantly decreased. Consistently, in these hard-to-reprogram cells, the levels of DNA damages induced by expression of pluripotency factors failed to be cleared after 72 hours, in contrast to fibroblasts with good reprogramming efficiency. Consistently, suppression of homologous recombination activity through expression of shRNA for BRCA1 or RAD51 in primary fibroblasts dramatically decreased iPS formation efficiency. In conclusion, our results suggested that aging associated defects in pluripotency reprogramming involves DNA damage repair responses, suggesting the possibility of improving iPSC derivation processes through augmentation of DNA damage repair.

F-2177

NIH CONTROL IPSC LINES AND STANDARDIZATION

Guokai Chen[1], Joshua Hunsberger[2], Steven Becker[2], **Chengyu Liu**[1], Manfred Boehm[1], Mahendra Rao[2]
[A]National Heart, Lung, and Blood Institute, [B]NIH Center for Regenerative Medicine, [1]National Institutes of Health, Bethesda, MD, United States

Induced Pluripotent Stem Cells (iPSCs) are the potential foundation of limitless source material for research and its applications. With iPSC technology evolving quickly due to technical improvements and new strategies, iPSC cell lines have increasingly different derivation and maintenance histories, making it difficult to compare and interpret results. NIH CRM and NHLBI met this challenge by developing control iPSC lines that could be widely used by academic and commercial entities. These control lines have been thoroughly characterized, and will set the benchmark for iPSC research. These control lines can be used for cell culture quality control and protocol development, as well as to generate lineage-tracking lines for basic research and drug discovery. These NIH control lines will be essential tools for translational research.

F-2178

HUMAN INDUCED PLURIPOTENT STEM CELLS FROM PATIENTS WITH RAI1 AND GRIN2B MUTATIONS

Yekaterina Galat[1], Mariana Perepitchka[1], Barbara Burton[2], Joel Charrow[2], Philip Iannaccone[1], Vasiliy Galat[1]
[A]Developmental Biology, [B]Division of Genetics, Birth Defects and Metabolism, [1]Lurie Children's Research Center, Northwestern University Feinberg School of Medicine, Chicago, IL, United States

Thus far, there has been very limited work exploring cellular mechanisms of neural dysfunction as a consequence of Smith-Magenis Syndrome SMS (del 17p11.2) or GRIN2B. SMS is a rare multisystem syndrome, caused by a chromosomal deletion or mutation in the RAI1 gene, which includes multiple congenital defects. A remarkable hallmark of the phenotype is an inverted pattern of plasma melatonin levels that contributes to sleep disorders. The melatonin inversion is not due to inversion of the central clock but to alteration of the regulation of hormone release regulatory elements. GRIN2B encodes the N-methyl-D-aspartate (NMDA) receptor subunit NR2B. The NMDA receptor channel has been shown to be involved in long-term potentiation, an activity-dependent increase in the efficiency of synaptic transmission thought to underlie certain kinds of memory and learning. The NR2 subunit acts as the agonist-binding site for glutamate. This receptor is the predominant excitatory neurotransmitter receptor in the mammalian brain. Here, we present the successful generation and characterization of a novel patient specific iPSC model for SMS and GRIN2B mutations both of which manifest in a complex phenotype including mental retardation. This model provides a unique opportunity to induce neuronal differentiation in stem cells from siblings that differ in single dominant mutations critical to normal neuronal differentiation. The model was established using a non-integrating Sendai virus reprogramming system. The generated iPSCs display embryonic stem cell-like morphology, express stem cell markers, and are capable of differentiation to three germ lineages. We further differentiated the iPSC lines to neural progenitors. We believe that by comparing gene expression data from mutant iPSCs, to "control" iPSCs and their corresponding neuronal derivatives it will be possible to discover mutation related alterations that can then be linked to impaired human neuronal development and provide a basis for discovery of novel therapeutic targets. These models present a unique tool for neurophysiology studies.

F-2181

ETHNICALLY DIVERSE PLURIPOTENT STEM CELLS FOR DRUG DEVELOPMENT

Eyitayo S. Fakunle[1], Victoria L. Glenn[1], John P. Schell[1], Thomas Touboul[1], Ha T. Tran[1], Gulsah Altun[1], Candace L. Lynch[1], Sara Abdelrahman[1], Cody Smith[2], Louise Laurent[3], Suzanne E. Peterson[1], Jeanne F. Loring[1]

[1]Chemical Physiology, The Scripps Research Institute, La Jolla, CA, United States, [2]CalAsia Pharmaceuticals, San Diego, CA, United States, [3]Department of reproductive medicine, University of California San Diego, La Jolla, CA, United States

Drug development is a lengthy and expensive process that can last more than 10 years and can cost in excess of $500 million. One reason for these exorbitant costs is “post-marketing drug failure,” in which drugs are recalled after hundreds of millions of dollars have been spent getting the drugs to market. The primary reason for post-marketing drug failure is idiosyncratic drug induced liver injury (DILI). There is extensive evidence showing that DILI is often due to variations in genes encoding drug-metabolizing enzymes that affect drug toxicity and efficacy. However, there is currently no practical means to screen candidate drugs in vitro for genome variation-associated toxicity early in the drug development pipeline. Current methods use in vitro testing, animal studies and human clinical trials, all of which are suboptimal for capturing population-based genetic variation. We propose that the development of a human induced pluripotent stem cell (iPSC) biobank that captures the most common genetic variations in drug metabolizing enzymes can provide the pharmaceutical industry with a renewable source of cells for early stage toxicology screens. We are building an ethnically diverse panel of iPSCs that can fulfill this need. We derived iPSCs from fibroblasts and keratinocytes obtained from skin biopsies and hair samples (outer root sheath of hairs) from a multiethnic cohort of healthy individuals, including several Caucasians, African Americans, a Yoruba individual, and other ethnicities. The Yoruba, from sub-Saharan Africa, are one of the original four ethnic groups analyzed by the worldwide haplotype mapping (HapMap) consortium. These iPSCs have been extensively characterized and genotyped by whole genome single nucleotide polymorphism (SNP) analysis. This data has allowed evaluation of loci involved in inter-individual and inter-ethnic differences in drug metabolism and toxicity. Since ninety percent of drug metabolism occurs in the liver, we are developing methods for directing differentiation of iPSCs into hepatocyte-like cells that can be incorporated into toxicity assays in order to determine the phenotypic correlates of the genetic variations we have found. We have differentiated iPSCs using a multi-step protocol, and have obtained cells that express typical hepatocyte markers, including ASGR, albumin and CYP1A2. We are characterizing the effect of common genetic variants with colorimetric assays (MTT, SRB, LDH) using common liver cell lines, with the aim of future transfer into assays using iPSC-based hepatocyte platforms. In addition we are integrating data from commercially available web-based direct-to-consumer personalized genomics services to enrich the information provided by iPSC genomics. Participants who donated samples for the diversity biobank also sent samples to a consumer genomics service. The service annotated genotypes for common phenotypic traits, disease risks and drug sensitivities, and web-based surveys add to the genotype/phenotype associations. We cross-validated the data by comparing SNP genotypes from the consumer service with data from our lab's analysis of the iPSCs. We found that web-based genomics services are an effective format to collect genotype and phenotype information from a large re-contactable cohort. Though this project is ongoing, we hope that it will lead to more efficient toxicity screening for drug development.

F-2182

CYTOGENETIC ABERRATIONS IN HUMAN ESC AND IPSC LINES SHOW SIGNIFICANT DIFFERENCES: A COMPARISON OF CHROMOSOME CHANGES IN 3816 IPS AND 2280 ES CELL LINES OVER A SEVEN YEAR PERIOD

Jared M. Finger, Stephanie A. Hazelbauer, Lorraine F. Meisner, Julie A. Johnson
Cell Line Genetics, LLC, Madison, WI, United States

We report cytogenetic findings based on the study of 2280 human ESC lines and 3816 human iPSC lines performed over a seven year period (2006-2012). Although both types of cell lines demonstrated a similar chromosome aberration rate (26% ESC, 21% iPSC), the types of aberrations were significantly different, suggesting that they arose by different mechanisms related to their respective methods of derivation. Under optimal conditions, ESC lines are derived from the inner cell mass of karyotypically normal embryos, while iPSC lines are derived from karyotypically normal adult cells that have been reprogrammed using integrating and non-integrating methods. In addition to inducing pluripotency, the reprogramming process can also induce random chromosome breakage and rearrangement. Although ESC lines initially have normal karyotypes, it has been shown that many develop numerical chro-

mosome abnormalities over time in culture (predominantly trisomy 12p, 17q, 1q, and 20q). These specific trisomies demonstrate a proliferative advantage that enable trisomic clones to overgrow a coexisting karyotypically normal cell line in relatively few passages. Our data will show that half the karyotypically abnormal ESC lines demonstrated numerical chromosome aberrations and slightly less than half showed structural chromosome aberrations (the majority of which were unbalanced translocations). In contrast, iPSC lines demonstrated few numerical aberrations, while the majority of the aberrations consisted of structural rearrangements (half of which were balanced translocations). Interestingly the majority of the balanced translocations detected in iPSC lines were observed at early passages without a coexisting second clone, while balanced translocations in ESC lines were rare. The fact that the structural aberrations (including balanced translocations) detected in iPSC lines involve different chromosomes in different cell lines and were detected at an early passage, suggests that they result from double strand DNA breakage and repair occurring during the reprogramming process. This is supported by the fact that the majority of the aberrations in iPSC lines are detected in all of the cells and are not found coexisting with a karyotypically normal clone (as is often observed with the emergence of trisomy 12p, 17q, 1q, and 20q in hESC lines). Our data will compare the different types and frequencies of chromosome aberrations in the ESC and iPSC lines.

F-2183

Isolation, Culture and Transfection of Human Keratinocytes : Application for Cell and Genetic manipulation

Fardin -. Fathi
Cellular and Molecular Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran, Islamic Republic of

Keratinocytes, particularly human keratinocytes, are difficult cells to isolation and grow. Reproducible isolation of epidermal keratinocytes from human is critically needed but most techniques have been complex and unreproducible. Human keratinocytes could be used to repair damaged skin, tissue engineering, gene therapy and recently iPS generation . In this report, based on previously published methods, we describe in detailed a simplified ,modified and reproducible method to isolate keratinocyte from human foreskin . we developed improved conditions for efficient transfection of the cells by testing of electroporation and lipofection methods and using of GFP plasmid vector. The keratinocyte cultures were established by enzymatically separating keratinocytes from human foreskin. The cells are allowed to attach on cell culture dishes coated by type I collagen , fibronectin, laminin or gelatin. Unattached cells were discarded and the attached cells were cultured in three types of serum-free medium and one type serum-based medium. The isolated cells showed the typical keratinocyte morphology and expressed the epithelial cell specific antigen, keratinocyte 14. Collagen type 1 , epilife medium and lipofection had the best results for isolation and transfection of cells. human keratinocyte-derived induced pluripotent stem cells were obtained in a gradual process , during the 2-3 weeks by using of Plasmid vector containing two self renewal genes. The results of this study shows that our described protocol can be used as a reproducible, simple and efficient method for isolation, cultivation and genetic manipulation of human keratinocytes which may be applicable in cell and gene therapy .

F-2184

DEVELOPMENT OF DIFFERENTIATION METHODS FROM HUMAN IPSCS/ESCS INTO NEPHRON PROGENITOR CELLS

Takafumi Toyohara[1], Yukiko Yamagishi[2], Shin-Ichi Mae[1], Tatsuyuki Inoue[1], Toshikazu Araoka[1], Tomoko Kasahara[1], Shinya Yamanaka[1], Hidenori Nakajima[2], Kenji Osafune[1]
[1]Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan, [2]Drug Discovery Research, Astellas Pharma Inc, Tsukuba, Japan

Nephron is the basic structural and functional unit of the kidney, which regulates the concentration of water and soluble substances within the animal body. In renal failure state, the nephrons are damaged and lose their functions. The generation of nephron progenitor cells from pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), can be available towards the development of regenerative medicine

for kidney diseases. However, the differentiation method from pluripotent stem cells into nephron progenitors remains to be developed. Nephron progenitor cells exist in the metanephric mesenchyme derived from an embryonic layer, intermediate mesoderm (IM). A transcription regulator *Osr1* is known to be one of the specific markers for IM. Recently, we have generated reporter human iPSC lines that contain an allele of *OSR1* into which a *green fluorescence protein* (*GFP*) gene was knocked-in by homologous recombination using bacterial artificial chromosome (BAC)-based vectors (OSR1-GFP knock-in hiPSC lines). We have also established the robust differentiation protocol for inducing human iPSCs/ESCs into OSR1(+) IM cells.
In this study, we aim to develop differentiation methods from human iPSC-derived OSR1(+) IM cells into nephron progenitors. Recent studies demonstrated that a transcriptional regulator *Six2* is one of the key molecules to maintain a progenitor state of nephron progenitors. In order to monitor and purify SIX2(+) nephron progenitor cells, we have generated double reporter human iPSC lines that are OSR1-GFP knock-in hiPSC lines containing an allele of *SIX2* into which a *tdTomato* gene was knocked-in by homologous recombination (OSR1-GFP/SIX2-tdTomato double knock-in hiPSC lines). We have also established a differentiation protocol for inducing human OSR1(+) IM cells into SIX2(+) cells using combinational treatment of growth factors, which produces up to 30% SIX2(+) cells. These cells expressed other marker genes for nephron progenitors, including CITED1, EYA1, WT1, SALL1, INTEGRIN ALPHA 8, HOXA11 and HOXD11. The human SIX2(+) cells could differentiate into multiple cell types included in the renal vesicle and the nephron *in vitro*, such as glomerular podocytes and proximal renal tubular cells. Furthermore, by coculture with mouse metanephric rudiments or embryonic spinal cord in organ culture settings, the human SIX2(+) cells reconstituted three-dimensional tubular structures. After implantation into mouse epididymal fat pad, the human SIX2(+) cells also formed three-dimensional tubular structures *in vivo*. These results suggest that our differentiation protocol can induce human iPSCs/ESCs into SIX2(+) nephron progenitor cells with similar developmental potential to that in embryos. The human iPSC-derived SIX2(+) cells may be used as cell sources to develop the regenerative medicine strategies for kidney diseases.

F-2185

THE CREATION OF A NEW HIGH THROUGHPUT PLATFORM FOR IPSC GENERATION AND CHARACTERISATION AT THE WELLCOME TRUST SANGER INSTITUTE

Chukwuma A. Agu, Alex Alderton, Ludovic Vallier, Richard Durbin, Chris Kirton
Wellcome Trust Sanger Institute, Cambridge, United Kingdom

The Wellcome Trust Sanger Institute (WTSI) is one of the world's leading genomic research centres with a mission to use genome sequences to advance understanding of the biology of humans and pathogens in order to improve human health. To exploit recent advances in the techniques used to create induced pluripotent stem cells (iPSC) the WTSI has embarked on an ambitious plan to create a centre of excellence for iPSC generation and phenotyping at its site in Hinxton, UK. This new centre of cellular generation and phenotyping (CGaP) will utilise state of the art automation and high throughput technology to manufacture iPSC on an industrial scale for subsequent academic and commercial exploitation. Scale-up technology will incorporate a number of established platforms such as liquid handling robots for maintenance and passaging of IPS cells and high-throughput cell imaging for cellular phenotyping. In addition we also plan to develop in-house automation for IPSC colony picking as well as taking advantage of the WTSI established sequencing automation.
One of the first projects to be supported in the new CGaP core facility will be the UK HipSci consortium led by Durbin *et al*. (www.hipsci.org). In the first phase of this project we plan to generate iPS cells from over 500 healthy and 500 disease-associated individuals over a period of 4 years. We will use these cells to discover how genomic variation impacts on cellular phenotype and identify new disease mechanisms.
The current approach in this project is to derive fibroblasts from 2mm skin biopsy samples, and then reprogram the fibroblasts using a non-integrating incompetent Sendai virus vector encoding the Yamanaka reprogramming factors: Oct4, Sox2, Klf4 and c-myc. Three clonal candidate iPS cell lines will be generated per donor and characterized. One or two iPS lines that pass QC will be selected for further study. Quality control (QC) characterisation will include screening for genomic integrity by Genotype array Chip, RNA gene expression assays, switch-off of reprogramming factors, expression of pluripotency genes; capacity for self-renewal; and ability to differentiate into

the three germ layers (ectoderm mesoderm and endoderm). Differentiation will be quantitated by immunostaining using an automated high throughput imaging. Lines selected that pass QC will undergo further genomic characterisation by Exome and RNA sequencing, DNA Methylation (450K) assays, FAIRE or DNAse1 and histone mark ChIP sequencing. Further cell biological assays will be undertaken in King's College London and at the WTCGRE in Dundee. The data acquired from the above assays including the QC data will be made available via a database held by the European Bioinformatics Institute (EBI). From each cell line a master cell bank will be derived and made available for distribution by a third party to the scientific community.

F-2186

MODEL SYSTEM FOR METHODICAL DISSECTION OF 3D MULTICELLULAR INTERACTIONS

Kelly R. Stevens[1], Mark D. Ungrin[2], Robert E. Schwartz[1], Kathleen S. Christine[1], Peter W. Zandstra[3], Christopher S. Chen[4], Sangeeta N. Bhatia[1]

[1]Harvard-MIT Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA, United States, [2]Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada, [3]Institute for Biomaterials & Biomedical Engineering, University of Toronto, Toronto, ON, Canada, [4]Department of Bioengineering, University of Pennsylvania, Cambridge, PA, United States

The safe and efficacious use of pluripotent stem cells in three-dimensional (3D) engineered tissue therapies necessitates comprehensive understanding and and control over human development. However, although multi-factorial signals (e.g., cell contact, paracrine signals, extracellular matrix) are known contribute to the development of tissues such as the liver bud, methodical dissection of their relative contributions in 3D settings has been hampered by the complexity of traditional animal model systems. Next generation model systems may enable methodical dissection of multicellular interactions in a variety of matrix environments. Here, we describe a novel 3D model system in which we combine multicellular 3D micropatterning in biomaterials, genetic reporter systems, high-resolution 3D imaging, and automated image analysis. This system enables precise microscale organization of many cell types, including induced pluripotent stem cell (iPS)-derived progeny, within a variety of synthetic and natural extracellular matrices and across tissues of sizes appropriate for in vitro, pre-clinical, and clinical biologic studies. We then used this system to dissect the functional impact of multicellular geometrical conformations between human induced pluripotent stem cell-derived hepatocyte-like cells (iHeps) or primary adult human hepatocytes and non-parenchymal cells. Patterning endothelial cells in a compartmentally distinct manner relative to adult hepatocytes ('paracrine conformation') significantly enhanced albumin secretion, a surrogate measure of hepatic function, compared to patterning endothelial cells directly adjacent to hepatocytes conformation ($*p < 0.05$). Similarly, patterning of iHeps and endothelial cells in paracrine conformation trended towards enhanced hepatic function. Next, stromal fibroblasts were patterned in a compartmentally distinct lattice compared to iHeps ('paracrine' conformation'), adjacent to iPS-Hep aggregates that had already compacted ('juxtaposed' conformation), or directly into microwells with iPS-Heps to create a heteroaggregate ('interpenetrating' conformation). Direct patterning of stromal cells to form heteroaggregates ('interpenetrating') resulted in significantly enhanced hepatic function compared to other conformations ($*p < 0.05$). Taken together, these results demonstrate that the structural organization of endothelial and stromal cells in 3D tissues modulates the degree to which they support and stabilize hepatic parenchyma. Our studies suggest that this system may be useful in future studies that probe differentiation state or signaling pathway activity of stem cell progeny or engineered tissue systems.

F-2187

MODELING α-1-ANTITRYPSIN DEFICIENCY USING PATIENT-DERIVED IPSC GENERATED HEPATOCYTES

Edgar Tafaleng[1], Bing Han[1], Pamela Hale[5], Alejandro Soto-Gutierrez[2], Masaki Nagaya[1], Carol Feghali-Bostwick[3], Darrell Kotton[6], Stephen Duncan[7], Donna Beer-Stolz[4], Stephen Strom[2], Jayanta Roy-Chowdhury[8], David Perlmutter[5], Ira Fox[1]

[A]Department of Surgery, [B]Department of Pathology, [C]Department of Medicine, [D]Department of Cell Biology, [1]University of Pittsburgh School of Medicine, Pittsburgh, PA, United States, [2]Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, United States, [3]Department of Medicine, Boston University School of Medicine, Boston, MA, United States, [4]Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI, United States, [5]Department of Medicine, Albert Einstein College of Medicine, New York, NY, United States

Classical α-1-antitrypsin deficiency (ATD) is the most common cause of hereditary pediatric liver disease. The disorder results from the PIZZ mutation in the protease inhibitor gene that alters the structure of α-1-antitrypsin (AT) and renders it aggregation-prone. This leads to the accumulation of the mutant AT variant within hepatocytes, causing hepatic injury and/or carcinogenesis through a toxic gain-of-function mechanism, and pulmonary emphysema through a loss-of-function mechanism. Although the PIZZ genotype is necessary for the development of ATD-mediated liver disease, there is significant variability in liver disease onset and severity among PIZZ individuals. The prevailing hypothesis is that other yet to be identified genetic and/or environmental factors affect liver disease onset and severity in PIZZ individuals. While it would be ideal to perform analyses of ATD-mediated liver disease in 1° hepatocytes bearing the human condition, these samples are limited and not readily available. Moreover, long-term culture and cryopreservation of hepatocytes lead to reduced function and viability. Induced pluripotent stem cells (iPScs) from ATD patients could provide an unlimited source of hepatocytes for disease modeling and drug testing. We generated 15 iPScs from ATD patients with liver disease of varying severity. Pluripotency marker expression and teratoma formation by the ATD iPSc were comparable with those by H1 human embryonic stem cells. Genomic DNA sequencing confirmed that all ATD iPSc lines carry the PIZZ mutation. We differentiated an iPSc line from an ATD patient with severe liver disease (severe LD), 2 iPSc clones from an ATD patient with lung disease but mild liver disease (mild LD), and a wild-type control iPSc line into hepatocytes. ATD and control iPSc-derived hepatocytes exhibited double nuclei, lipid droplets, glycogen rosettes, well-developed bile canaliculi with apical microvilli, desmosomes, and tight junctions by transmission electron microscopy. Similar to that seen in ATD patient liver biopsies, electron micrographs of ATD iPSc-derived hepatocytes showed poorly organized and dilated rER. We also found that although the differentiated iPScs secreted similar levels of albumin, mild LD iPSc-derived and severe LD iPSc-derived hepatocytes secreted only 75% and 55% of the AT levels secreted by control iPSc-derived hepatocytes. Using pulse-chase analysis, we detected a slower rate of disappearance of intracellular AT in ATD iPSc-derived hepatocytes (t½= 2.1 to 4 hr) compared to control cells (t½= 1.3 hr). More importantly, we found that the delayed disappearance of intracellular AT observed in ATD iPSc-derived hepatocytes is more apparent in cells obtained from the patient with severe liver disease (t½= 4 hr) compared to those from the patient with no liver disease (t½= 2.1 to 2.2 hr). This result is consistent with results obtained from 1° hepatocytes of an ATD patient with severe liver disease (t½= 4.2 hr). There was no significant difference between the rate of disappearance of intracellular AT between hepatocytes derived from the two iPSc clones from the mild LD patient suggesting that the result is not an artifact of reprogramming or differentiation. This is the most detailed demonstration of the pathobiological defect seen in ATD in an iPSc-derived hepatocyte model. Using these ATD iPSc-derived hepatocytes, we plan to identify modifier genes that affect the severity of liver disease in PIZZ individuals.

F-2188

EFFECT OF LYSOPHOSPHATIDYLCHOLINE ON DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS INTO CARDIOVASCULAR PROGENITOR CELLS

Toshiaki Ishizuka, Ayako Ozawa, Hazuki Goshima, Yasuhiro Watanabe
National Defense Med Coll, Tokorozawa, Japan

[Introduction] It was reported that gene transfer of human apolipoprotein A-I, a main component of high density lipoprotein, may enhance cardiac differentiation of human induced pluripotent stem (iPS) cells. On the other hand, it was shown that oxidized low-density lipoprotein (ox-LDL) inhibits differentiation of rat bone marrow stem cells or endothelial progenitor cells into endothelial cells. However, it remains unknown that treatment with ox-LDL affects differentiation of human iPS cells. In the present study, we examined whether stimulation with lysophos-

phatidylcholine (lysoPC), a main component of ox-LDL, regulates cardiovascular differentiation of human iPS cells. **[Materials and Methods]** To induce cardiovascular differentiation of human iPS cells, the cells were cultured on feeder cells in serum-free medium with bFGF (1.6 nM), BMP-4 (1.8 nM), Activin (0.1 nM), and VEGF (0.6 nM) for 10 days. Simultaneously,the undifferentiated cells were treated with lysoPC (0.1 ~ 10 μM) or SB203580 (a p38MAPK inhibitor; 1 μM). The differentiation potential from human iPS cells into cardiovascular progenitor cells was evaluated by VEGF type 2 receptor (VEGFR2) or α-smooth muscle actin (α-SMA) expression using immunofluorescence staining or western blot analysis. **[Results]** The stimulation with lysoPC (3 μM) significantly enhanced VEGFR2 or α-SMA expression. The effect of lysoPC was significantly inhibited by pretreatment with SB203580. We also confirmed that the VEGFR2-positive cells derived from human iPS cells can differentiate into vascular endothelial cells or vascular smooth muscle cells. **[Conclusion]** These results suggest that stimulation with lysoPC may enhance the differentiation of human iPS cells into cardiovascular progenitor cells via p38MAPK signaling pathway.

F-2191

INTEGRATING DYNAMIC MICRORNA EXPRESSION AND EPIGENETIC CONTROL DURING SOMATIC CELL REPROGRAMMING

Andrew Corso[1], Peter Tonge[1], Jennifer Clancy[2], Hardip Patel[2], Mira Puri[1], *. International PG consortium[1], Thomas Preiss[2], Andras Nagy[1]

[1]Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, Toronto, ON, Canada, [2]Genome Biology Department, The John Curtin School of Medical Research, Canberra, Australia

Induced pluripotent stem cells (iPSCs) are promising therapeutic candidates for the future of regenerative medicine. Not only do they resemble both the self-renewal and pluripotent properties of embryonic stem cells (ESCs), but they also bypass the problem of immune rejection of potential ESC therapies. Before iPSC technology can be considered for clinical therapies, a thorough understanding of the underlying molecular mechanisms of reprogramming is necessary. MicroRNA (miRNA) has already been shown to influence the reprogramming process, yet the function of the majority of miRNA is still unknown.

Multiple primary iPSC cultures were derived from wildtype mouse embryonic fibroblasts (MEFs) via the addition of four inducible transgenic factors (c-Myc, Klf4, Oct4, and Sox2). Twenty-eight clonal cell lines have been characterized in depth (Tonge et al., ISSCR presentation) to show that primary iPSC cell lines cluster into two distinct groups (type-A and type-B iPSCs). Whilst morphologically and molecularly distinct from each other, both types exhibit pluripotent properties and are stable under transgene expression.

In order to model these distinct iPSC states, a unique dataset was generated using an inducible secondary reprogramming system. Here, transgenic expression of the four reprogramming factors triggers population-based reprogramming of secondary MEFs via the addition of doxycycline. This system effectively models the progression of somatic cell reprogramming to both type-A and type-B iPSC states, and allows for a population-based molecular analysis through a variety of OMICS platforms, including transcriptome sequencing, epigenetic analysis, and small RNA deep sequencing.

By comparing this small RNA sequencing data from type-A iPSCs and type-B iPSCs, we found that miRNA expression profiles during reprogramming can be correlated with the surrounding epigenetic architecture, consisting of histone modifications (H3K4me3, H3K27me3, and H3K36me3) and CpG methylation. Furthermore, type-A iPSCs maintain epigenetic marks of the parental fibroblasts that influence miRNA expression. This provides valuable insight into the mechanisms of expression/repression for each of the candidate miRNAs.

We then identified miRNAs which are either significantly up- or down-regulated between type-A and type-B iPSCs. These candidate miRNAs were cloned into doxycycline-inducible plasmids and transfected, along with the reprogramming factors, into primary MEFs. Each of the candidate miRNAs has been characterized based on the effects that they have on reprogramming, including efficiency and iPSC state.

Overall, by examining the differences in miRNA expression between type-A and type-B iPSCs, we have identified several miRNAs that play a previously unknown role in the process of reprogramming. In many cases, the surround-

ing epigenetic environment can account for the expression of these miRNAs. This has allowed us to more clearly understand the important role of miRNAs during reprogramming of somatic cells.

F-2192

GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM DOMESTIC GOATS- CAPRA HIRCUS

Bhanu Prakash V. Telugu[1], Anne Powell[2], Shelley Swing[1], Neil Talbot[2], Le Ann Blomberg[2], David M. Donovan[2]
[1]Animal and Avian Sciences, University of Maryland, Beltsville, MD, United States, [2]Animal Biosciences and Biotechnology Laboratory, USDA, ARS, Beltsville, MD, United States

The creation of genetically modified (GM) goats provides a powerful method for improving animal health, enhancement of production traits, animal pharming, and ensuring food safety, all of which are high priority goals for animal agriculture. However, GM goats, and the GM livestock field in general, has languished without significant technological gains for the past two decades due to the lack of authentic goat embryonic stem cell (ESC) lines that revolutionized the GM mouse field. In goats, fibroblasts are routinely modified to carry the intended genetic change and then used as nuclear donors in nuclear transfer (NT) to generate GM offspring. But this technique has several drawbacks; the process is inefficient, pregnancy losses are high, and neonates exhibit developmental abnormalities. Also, and critically, fibroblasts cannot be maintained in culture sufficiently long enough to make, select for, and molecularly characterize site-specific or targeted genetic modifications, i.e., gene knock-outs or knock-ins. Accordingly, the availability of goat ESC lines that are characteristically immortal in culture would be of enormous benefit for creating GM animals. As an alternative to long sought after goat ESC lines, we have generated goat induced pluripotent stem cells (giPSC) lines by forced expression of OCT4, SOX2, MYC, KLF4, LIN-28 and NANOG reprogramming factors (RF) in combination with a MIR302/367 cluster, all obtained from another ruminant, bovine, and delivered by cleavable (floxed) lentiviral vectors (LV). In order to minimize integrations, the RF genes were assembled with porcine teschovirus-1 2A (P2A)-self cleaving peptides that facilitate tri-cistronic expression from each lentiviral vector. Following transduction with the LV vectors, the cells were cultured in a semi-defined serum-free medium (KOSR) containing FGF and/or LIF on irradiated feeder cells consisting of either mouse embryonic fibroblasts or SNL cells. The SNL are specialized mouse fibroblast STO cell lines (S) that are neomycin resistant (N) and express murine LIF (L). The resulting giPSC exhibit cell and colony morphology typical of human ESC, i.e., well defined borders and a high nuclear to cytoplasmic ratio, have a short cell-cycle interval, and are alkaline phosphatase positive- a marker of pluripotent cells, and able to generate teratomas. Currently, the levels of expression of endogenous pluripotency-associated genes in the giPSC are being assessed by deep sequencing, along with their ability to differentiate into directed lineages in vitro. [This work is supported by ARS (CRIS 1245-31000-103-00D), UMD MAES seed grant and Investigator funds from Department of Animal and Avian Sciences to BT].

F-2192

IPSC-DERIVED MTDNA DISEASE MODEL

Riikka H. Hämäläinen, Tuula Manninen, Hanna Koivumäki, Timo Otonkoski, Anu Suomalainen
Univ of Helsinki, Helsinki, Finland

Mitochondrial DNA (mtDNA) mutations are a common cause of inherited metabolic disorders, and manifest with vast clinical heterogeneity. The molecular basis of this variability is mostly unknown, as the lack of model systems has hampered mechanistic studies. We generated induced pluripotent stem cells (iPSC) from patients carrying the most common human mitochondrial disease mutation in mtDNA, m.3243A>G, and further differentiated these to several identifiable tissue types in teratomas and to neuronal cells in vitro. During reprogramming, heteroplasmic mtDNA showed specific segregation patterns, along with concomitant changes in mtDNA amount and cellular organization, mimicking those during epiblast specification. iPSCs differentiated to neurons and teratomas manifested cell-type specific respiratory chain deficiency patterns. Complex-I defect predominated in differentiated cells, mimicking the phenotype seen in MELAS patient tissues. Our data show that iPSCs provide an excellent tool to elucidate mechanisms of mtDNA segregation and a source of heteroplasmic human tissues to be used as disease models.

F-2194

COMPARATIVE ANALYSIS FOR TRANSCRIPTOMES OF IPS CELLS AND PREIMPLANTATION EMBRYOS IN PIG <!--ENDFRAGMENT-->

Suying Cao[1], Jianyong Han[1], Jun Wu[2], Ning Li[1]
[1]State Key Laboratories for Agrobiotechnology, China Agricultural University, Beijing, China, [2]Novogene Bioinformatics Technology Co.Ltd, Beijing, China

To date, there is no stable pig iPS cells (induced pluripotent stem cells) which contain the same characteristics of mouse iPS cells, especially regarding germ line transmission. Incomplete epigenetic reprogramming of the somatic cells may be a reason for developmental failure of iPSCs to generate germ-line transmission in pigs. This is mainly due to current in vitro culture conditions, which are not suitable for the maintenance of true pig ES cells. So there is no good transcriptome standard to evaluate the current pig iPS cells. The ICM (inner cell mass) from blastocyst may be positive control which can replace pig ES cells to evaluate pig iPS cells. We selected two pig iPS cell lines for transcriptome analysis compared with those of pig putative ICM (pICM) and different stages of embryos. The iPS cells exhibit good ES cell-like morphology, are alkaline phosphatase-(AP-) positive and can form teratomas. Cluster correlation analysis showed the pig iPS cells are more similar to morula than any other stage of the embryos. However, 61% of pICM genes were shared with that of piPS cells. These suggest that the iPS cells share some characters with ICM and morula. We therefore analyzed the common genes of the ICM and morula and compare them with that of the pig iPS cells. GO analysis for these common genes suggested that they share pluripotency-related pathways, like p53 signaling pathway, mTOR signaling pathway, Insulin signaling pathway, Notch signaling pathway, TGF-beta signaling pathway, and Wnt signaling pathway. Pig ICM, morula, and pig iPS cells shared 22.4% of their genes, which belong to pathways related to the common cell biology (like that cytoplasm, nucleus, mitochondrion, cell cycle). Our results represent a significant step in characterizing pig preimplantation embryos and pig iPS cells and provide a resource for pig pluripotent stem cell engineering.<!--EndFragment-->

F-2195

MELANOCYTES DERIVED FROM THE TRANSGENE-FREE HUMAN INDUCED PLURIPOTENT STEM CELLS

Jennifer C. Jones[1], Karen Sabatini[1], Xiaoyan Liao[1], Ha T. Tran[1], Candace L. Lynch[1], Robert E. Morey[1], Francesca S. Boscolo[1], Jason Stein[2], Ying Liu[3], Louise C. Laurent[4], Suzanne E. Peterson[1], Jeanne F. Loring[1], **Yu-Chieh Wang**[1]
[1]Department of Chemical Physiology, Scripps Research Institute, La Jolla, CA, United States, [2]California State University, San Marcos, CA, United States, [3]Department of Neurosurgery, University of Texas Health Science Center, Houston, CA, United States, [4]Department of Reproductive Medicine, University of California, San Diego, CA, United States

Defects in melanocytes have been implicated in the etiology of a variety of human skin disease and disorders. To establish a novel platform for studying melanocyte-associated disease and promote the development of a cell-based therapy for patients

with hypopigmentation disorders, we developed an efficient pipeline for derivation of transgene-free human iPSCs (hiPSCs) that were generated from two distinct types of cutaneous cells, followed by an effective *in vitro* directed differentiation method for generating high yields of functional melanocytes. Transgene-free hiPSCs were successfully obtained from human dermal fibroblast (HDF) cells as well as primary human melanocytes (HM), using a non-integrative reprogramming approach mediated by Sendai virus-based vectors encoding four transcriptional factors. The hiPSCs were positive for pluripotency-associated biomarkers and differentiated *in vitro* and *in vivo* into derivatives of all three germ layers. Using two newly-developed differentiation protocols, we obtained cells displaying morphology and pigmentation typical of melanocytes from both HDF- and HM-derived hiPSCs after 30 days of directed differentiation. MITF, a marker for melanocyte progenitors, was expressed in more than 90% of the differentiated derivatives at the end of the differentiation procedure. Other melanocytic biomarkers including PAX3, TYR, MART-1, TYRP1, SILV and SOX10 were also expressed in the differentiated derivatives. Genome-wide gene expression profiling revealed that the differentiated derivatives were closely clustered with HM cells and differed from all undifferentiated hiPSC samples. We demonstrated that the cells remained genomically stable during the reprogramming and differentiation process by comparing the differentiated derivatives of HM-derived iPSCs cells to the HM cells using a high-resolution SNP genotyping platform and copy number variation analysis. In the differentiated derivatives, melanogenesis was enhanced by α-MSH in a dose-dependent fashion, suggesting that these hiPSC-derived melanocytes accurately mimic the ability of *bona fide* melanocytes to respond to α-MSH. Additionally, our melanocytic derivatives integrated well into semi-autologous skin equivalents and showed immunocompatibility in a mixed lymphocyte reaction assay. In summary, genetically-stable, functional melanocytes with high purity can be efficiently obtained from transgene-free hiPSCs that were generated from different types of cutaneous cells. This system represents an unlimited source of custom human melanocytes that can be used in novel approaches for modeling human skin disease and to provide material for autologous transplantation.

F-2196

PATIENT DERIVED INDUCED PLURIPOTENT STEM CELLS AS A MODEL FOR TUBEROUS SCLEROSIS COMPLEX

Eric A. Armour[1], Bryan E. Cawthon[2], Aaron Bowman[2], Kevin Ess[2]
[A]Cell and Developmental Biology, [B]Neurology, [1]Vanderbilt University, Nashville, TN, United States

Tuberous Sclerosis Complex (TSC) is a multi-organ hamartomatous disease caused by loss of function mutations in either the *TSC1* or *TSC2* genes. Despite involvement of multiple organs such as the kidneys, lungs, and skin, neurological aspects are usually the most severe due to a very high prevalence of mental retardation, autism and epilepsy. The protein products of *TSC1* and *TSC2*, hamartin and tuberin respectively, regulate the mTOR kinase-signaling pathway. Current models of TSC suggest that hamartoma formation is secondary to a loss of heterozygosity at either the *TSC1* or *TSC2* loci, and subsequent hyperactivation of mTOR Complex 1. While this loss of heterozygosity has clearly been reported in other organ systems, second hit mutations in neural lesions have only rarely been identified. Thus, current research into TSC that employ animals with mutations in both copies of the gene may be an imprecise model for the human disease.
To begin to define the role of the human *TSC1* and *TSC2* genes during the pathogenesis of TSC, we have generated induced pluripotent stem cells (iPSC) from multiple patients with TSC. These patient derived iPSC will allow *in vitro* modeling of TSC to dissect abnormalities of proliferation, cell size, and neuronal differentiation. TSC patient derived iPSC are larger and proliferate faster compared to control lines. They also spontaneously differentiate to neurons, even under stem cell maintenance conditions. Sequencing revealed these patient derived lines are only heterozygous for *TSC1* or *TSC2* mutations. Western blot analysis shows protein products for both *TSC1* and *TSC2* further supporting heterozygous iPS lines. Interestingly, preliminary data show that mTOR signaling is still dysregulated, suggesting possible haploinsufficiency of the *TSC1* and *TSC2* genes as causing neurologic disease. Treatment with rapamycin, an inhibitor of mTOR Complex 1 signaling, slows proliferation and decreases the rate of spontaneous differentiation to that of control iPSC lines – further implicating dysregulated mTOR in the phenotype of these patient derived cells.

We are using directed differentiation to derive neurons from the iPSC lines. By comparing neural progenitor cells and mature neurons derived from patient iPSC to those from control iPSC derived from healthy volunteers, we expect to identify changes contributing to the neurological symptoms seen in TSC patients. Furthermore, to test whether haploinsufficiency and subsequent hyperactivation of mTORC1 is contributing to the neurodevelopmental defects of TSC, neurons will be derived from iPSC in the presence of rapamycin. Results from this directed differentiation will be confirmed in cortical samples removed during epilepsy surgery from the same patients that had a skin biopsy and generation of iPSC lines.
By combining *in vitro* results from patient derived iPSC from multiple patients with TSC and their *ex vivo* human brain samples, we expect to find novel pathological mechanisms and also closely define the underlying genetics and relationship of mTORC1 and mTORC2 signaling to abnormal neuronal differentiation.

F-2198

SURVIVAL OF MOTOR NEURON (SMN) REGULATES CELL FATE IN DIVERSE STEM CELL POPULATIONS

Maureen M. Sherry-Lynes, Natalia Rodriguez-Muela, Jesse Mull, Lee L. Rubin
Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States

Spinal muscular atrophy (SMA) is a childhood genetic disease caused by the loss or mutation of the Survival of Motor Neuron 1 (SMN1) gene. Loss of SMN expression leads to motor neuron degeneration, and SMA patients suffer from a progressive and often fatal motor impairment. For this reason, SMA has traditionally been considered a motor neuron disease; however, there is increasing evidence that SMN loss affects other tissues as well. SMA patients, for example, exhibit cardiac development defects which are mirrored in a mouse model of SMA. Because SMN is a ubiquitously expressed protein, we hypothesize that it may be important in cell types other than motor neurons, and that defects in other tissues may contribute to disease pathogenesis. We have shown that this is the case in satellite cells isolated from the severe SMA mouse model. SMN-deficient muscle satellite cells exhibit premature differentiation defects both in vitro and in vivo. The underlying cause is that SMN-deficient satellite cells give rise to myogenic progenitor cells more quickly than do wild type satellite cells, but these cells do not efficiently form multinucleated myotubes. Importantly, this effect is cell autonomous, suggesting that SMN loss affects satellite cells directly, rather than as a result of motor neuron degeneration. In order to determine whether the premature differentiation defects observed in muscle satellite cells extend to neuronal differentiation, we compared motor neuron production from wild type and SMN-mutant mouse embryonic stem cells. Surprisingly, we found that, when using our standard differentiation protocol, that the SMN-deficient stem cells give rise to motor neurons earlier than do wild type cells. However, a high percentage of these prematurely born motor neurons die abruptly, not even surviving the first ten hours of culture. Finally, we wanted to examine whether these differentiation defects observed in mice also apply to human cells. To address this question, we derived 35 iPS lines from 3 SMA patient samples using multiple reprogramming methodologies. We found that SMN-deficient iPS cells are prone to spontaneous differentiation in culture, and that the extent of this differentiation is tightly correlated with SMN level. In both pluripotency and neural differentiation conditions, SMA iPS cells lose the expression of pluripotency markers such as nanog and oct4 more rapidly than do wild type cells. Knockdown of SMN levels using a shRNA-containing lentivirus in wild type ES or iPS cells recapitulates the spontaneous differentiation phenotype, suggesting that the premature differentiation is due to loss of SMN expression, rather than variability between iPS lines. Together, these results suggest that the critical reduction in SMN that causes SMA might alter the fate of multiple stem cell populations and that there may be additional, still unappreciated, components of the disease in children.
Supported by grants from the Spinal Muscular Atrophy Foundation, the NINDS, and the Harvard Stem Cell Institute.

F-2201

CHARACTERIZATION OF GENE NETWORKS AT THE EARLY PHASE OF SOMATIC CELL REPROGRAMMING

Masamitsu Sone, Shinya Yamanaka, Takuya Yamamoto
CiRA, iCeMS, Kyoto University, Kyoto, Japan

Somatic cells can be reprogrammed into induced pluripotent stem (iPS) cells by introducing a few transcription factors. However, how these exogenous transcription factors activate the pluripotency gene network is largely unknown. Especially, it remains totally unclear what is the critical events just after over expression of reprogramming factors. To elucidate the molecular mechanisms that can direct the somatic cells toward reprogramming, we profiled the gene expression in mouse embryonic fibroblasts (MEFs) at early stages of iPS cell derivation.
First, we investigated the timing at which the somatic cells are committed to be reprogrammed into iPS cells. Surprisingly, we found that the cell fates are almost determined by as early as three days after retroviral introduction of reprogramming factors (Oct3/4, Sox2, Klf4, and Glis1) into p53-/- MEFs. Second, using BioMark high-throughput real time PCR system, we compared gene expression profiles at the early stages (day 3, 6) in the two types of cells, one is efficiently reprogrammed at the later stage (day 12) and the other is not. We identified several genes which are specifically expressed in the former cells, and demonstrate that some of these genes enhance the reprogramming efficiency when combined with Yamanaka factors (Oct3/4, Sox2, Klf4, and c-Myc). Finally, we investigated the transcriptional relationship among these reprogramming activators at the early stage (day4), and found that they connect to each other and make up a complicated transcriptional network that contains a lot of feedback and feedforward loops.
We are now planning to apply genome-wide transcriptome techniques to this network analysis to uncover the roles of these activators in somatic cell reprogramming process.

F-2202

CHEMICAL GENETIC IDENTIFICATION OF MOLECULES THAT POTENTIATE HEPATIC MATURATION OF A HUMAN IPS-DERIVED HEPATOCYTES BASED SCREENING SYSTEM AS A TOOL FOR LOOKING INTO THE MECHANISM OF HEPATIC MATURATION

Taiji Yamazoe[1], Nobuaki Shiraki[1], Motonari Uesugi[2], Kazuhiko Kume[1], Shoen Kume[1]
[1]*Inst of Molecular Embryology & Genetics, Kumamoto Univ., Kumamoto, Japan,* [2]*Institute for integrated cell-material science, Kyoto Univ., Kyoto, Japan*

High-throughput chemical screening strategy is widely used to search for molecules that play important roles in biological pathways. Chemical libraries that contain thousands of small molecular weight compounds are now available. However, to perform high-throughput screenings, a large number of cells are required, which might be a barrier in cell-based biology, particularly when human derived materials are required.
Lines of evidence have shown that ES and iPS cells recapitulate normal developmental processes, and suggest that ES and iPS cells provide attractive cell sources for routine access to large numbers of cells that enable the high-throughput screening.
Here, we established a high-throughput screening system to find molecules that promote hepatic differentiation of human iPS cells. We differentiated human iPS cells into the hepatic lineage for 19 days, and then challenged with chemicals.
We screened a chemical library, consisting over 1000 clinically used biological active pharmaceutical drugs. Based on the immunochemical analysis with hepatic markers, we obtained candidate chemicals that potentiated hepatic maturation. To apply ES/iPS cells in the process of drug development, it is necessary for the differentiated ES/iPS cell-derived hepatocytes to be able to show metabolic functions of the liver. We then selected chemicals that showed marked effects and checked whether the candidate drugs potentiated hepatic maturation of human iPS cell, by measuring the p450 enzymatic activity of CYP3A4, and ICG (Indo-cyanine-green) -uptake tests as an indicator for detoxification. ICG test with cells added with candidate drug showed a larger ICG-positive area than those in the control group. Luciferase assays were performed to evaluate p450 activity, which also showed higher levels of luminescence in cells that added with candidate drug. Moreover, we found that the target molecules of the chemical compound were expressed in a certain time window in differentiated human iPS cells. Finally, we examined their effects on murine hepatoblasts and human cancer cell lines, which were conventionally used in other screening systems. We found the candidate drugs were effective on potentiating differentiation of murine hepatoblasts into hepatocytes, thereby suggesting that the present mechanism of hepatic maturation could be common between mice and humans. However, human liver cancer cell lines did not turn off AFP expression upon treatment with the

candidate drugs. The quantitative PCR analysis revealed that these cancer cells had low expression levels of drug targets molecules. Therefore, it is possible that the cancer cell lines are not a good assay system, and that the candidate drug might be related with liver carcinogenesis. In conclusion, our screening system is useful in studies of the maturation mechanisms of human liver development. Because this screening system utilized human iPS cell, it enables us to identify molecules involved in hepatic maturation, which might be lacking in the cancer cell line.

Reprogramming

F-2203

THERAPEUTIC OPPORTUNITIES IN REGENERATIVE MEDICINE - HOW TO MAKE SAFE DRUGS

Patrik J. Andersson[1], Frank Seeliger[1], Gabriella Brolén[2], Björn Löwenadler[2], Ian Cotgreave[1]
[A]Global Safety Assessment, [B]Discovery Science, [1]AstraZeneca R&D, Mölndal, Sweden

One of the more obvious applications of increased stem cell biology understanding lies in the area of regenerative medicine. A strategic decision has been taken by AstraZeneca to complement the established cardiovascular areas with more novel regenerative medicine approaches. The current focus is not cell-based or gene transfer therapies, but rather “regenerative pharmacology”. This could be described as influencing local developmental biological processes by stimulating resident, endogenous stem and progenitor cells to proliferate and/or differentiate in a direction that would lead to tissue repair and normalization of function. It is quite likely that central signaling pathways need to be modulated to reprogram cells to achieve sufficiently efficacious treatment. This introduces new and quite significant challenges from a drug safety perspective. What, when and how should potential adverse effects be measured? There are several guidelines from e.g. FDA and EMA on various aspects of gene and cell therapy that describe regulatory expectations for clinical trials. Many of those principles could likely be applied also to a regenerative pharmacology approach. However, there is a strong need to understand potential safety concerns much earlier in a project than at the stage of clinical trials. One of the most important areas to develop is thorough understanding of the particular pharmacokinetic/pharmacodynamic relationship and the duration and periodicity of effect on the target developmental biological program. This requires relevant and specific biomarkers for both efficacy and safety that translate well and can be modeled across species. It also requires in vitro and in vivo models where those biomarkers and processes such as proliferation, de-differentiation and cellular migration ideally can be followed over time. If potential safety concerns related to the biology are identified, do we understand how to mitigate those risks? Are the safety departments in Pharma companies prepared for these challenges? Can we rely on currently established toxicological principles or is a new safety testing paradigm needed for regenerative pharmacology? Key aspects on how AstraZeneca plan to deal with these questions will be discussed.

F-2204

ACUTE REDUCTION OF OXYGEN TENSION ENHANCES THE INDUCTION OF NEURONS FROM HUMAN FIBROBLASTS

Jonathan Davila, Soham Chanda, Cheen Euong Ang, Thomas C. Südhof, Marius Wernig
Stanford University, Palo Alto, CA, United States

We and others reported recently the successful conversion of human fibroblasts into functional induced neuronal (iN) cells, however the reprogramming efficiencies were very low. More robust methods must be developed before iN cells can be used for translational applications such as disease modeling or transplantation-based therapies. Here, we describe a novel approach in which we significantly enhance iN cell conversion efficiency of human fibroblast cells by reprogramming under hypoxic conditions (5% O2). Fibroblasts were derived under high (21%) or low (5%) oxygen conditions and reprogrammed into iN cells using a combination of the four transcription factors BRN2, ASCL1, MYT1L and NEUROD1. Reprogramming efficiencies were ascertained by Map2 immunofluorescence 23 days post infection. Interestingly, the increase in the reprogramming capacity was only observed when fibroblasts experienced an acute drop in the O2 tension upon infection. Cells derived and reprogrammed under hypoxic conditions did not produce more iN cells. Importantly, the increase in reprogramming efficiency was robust in three independ-

ently derived cell lines. Immunofluorescence and electrophysiological analysis showed no effect on the quality and functional maturation of iN cells reprogrammed in 5% O2. Surprisingly, the acute drop in oxygen tension did not affect cell proliferation or cell survival and is not synergistic with blockade of GSK3β and Smad-mediated pathways. Our results show that lowering the O2 tension at initiation of reprogramming is a simple and efficient manner to enhance the production of iN cells which will facilitate their use for basic discovery and regenerative medicine.

F-2205

DIRECT REPROGRAMMING OF FIBROBLASTS INTO BEATING CARDIOMYOCYTE-LIKE CELLS WITH THE MYOD TRANSACTIVATION DOMAIN

Hiroyuki Hirai[1], Nobuko Kikyo[2], Nobuaki Kikyo[1]
[A]Stem Cell Institute, Department of Genetics, Cell Biology and Development, [B]Stem Cell Institute, Department of Neurology, [1]Univ of Minnesota, Minneapolis, MN, United States

Direct reprogramming of non-cardiac cells into cardiomyocytes provides a novel strategy to obtain cardiomyocytes for transplantation, drug screen and disease modeling. Fibroblasts can be directly reprogrammed to cardiomyocyte-like cells by introducing defined genes. However, the reprogramming efficiency remains extremely low, delaying clinical application of this strategy to regenerative cardiology. MyoD is a master regulator for skeletal muscle differentiation and is an exceptionally potent transcription activator. Recently we found that the fusion of MyoD transactivation domain to the pluripotency factor Oct4 can improve the efficiency of converting skin fibroblasts to induced pluripotent stem cells (iPSCs) more than 10- to 100-fold. We examined whether fusion of the MyoD transactivation domain to cardiac transcription factors would facilitate reprogramming of fibroblasts to cardiomyocytes. We fused the MyoD transactivation domain to Mef2c, Gata4, Hand2, Tbx5 and other genes and transduced the genes in various combinations with wild-type genes into mouse non-cardiac fibroblasts. Transduction of some of the combinationsproduced much larger clusters of beating cardiomyocyte-like cells faster than the combination of the 4 wild-type genes, with more than 15-fold greater efficiency of around 4%. This finding indicates that appropriate combinations of fusion of a powerful transactivation domain to heterologous factors can promotes the efficiency of direct reprogramming of fibroblasts into beating cardiomyocyte-like cells.

F-2206

NIH3T3 CAN BE FUSED EFFICIENTLY AND REPROGRAMMED AS PLURIPOTENT STEM CELLS BY DEMETHYLATION OF THE PROMOTER OF OCT4 AND NANOG

Guanghua Peng, Wei Wu, Haiwei Xu, Zheng Qin Yin
Third Military Medical University, Chongqing, China

NIH3T3 can be fused efficiently and reprogrammed as pluripotent stem cells by demethylation of the promoter of OCT4 and Nanog

Wei Wu[1,2], Haiwei Xu[1,2],Zhengqin Yin[1,2], Guanghua Peng[1,2*]
[1]Southwest Eye Hospital, Southwest Hospital, Third Military Medical University, Chongqing, 400038, PR China
[2]Key Lab of Visual Damage and Regeneration & Restoration of Chongqing, Chongqing , 400038, China
EMAIL: peng63105@yahoo.com

[Abstract] Objective To establish a high throughput cell electro-fuion platform and to fuse mouse embryonic stem cells(mESCs) with somatic cells, and to explore the mechanism of somatic cells reprogramming. **Methods** With the microfluid chip we designed, we fused the mESCs carrying GFP with NIH3T3 carrying RFP. We sorted the fused cells by flow cytometer (FACS) and detected the pluripotency of fused cells by qPCR, immunofluorescence, Western blot. We next investigate the methylation status of the OCT4 and Nanog promoters by dot bot, Bisulfite sequencing PCR(BSP) and glucMS-qPCR. In order to explore the mechanism of demethylation during the somatic cells reprogramming, we detect the expression of activation-induced cytidine deaminase (AID, also known as AICDA), ten eleven translocation (Tet) proteins, TDG and MBD4 by qPCR, immunofluorescence and Western blot. **Results** The

fused cells can form embryonic stem cell-like clone and embryoid bodies.The qPCR results show upregulation of pluripotent genes Nanog, Oct4 and downregulation of somatic marker genes LaminA, CKAP2 in the fused cells compared with NIH3T3, which is confirmed with the Western blot and immunofluorescence.Through dot blot, we found that NIH3T3 were high-methylated while those in the fused cells were low-methylated. Subsequently we found that the promoter of OCT4 and Nanog demethylated in the fused cells during cell fusion by BSP, which coincides with glucMS-qPCR. Afterwards, AID, Tet1 and Tet2 in the
fused cells were significantly upregulated compared with NIH3T3 by qPCR, immunofluorescence and Western blot.
Conclusion The mESCs and NIH3T3 can be fused efficiently with the microfluid chip, the somatic cells were reprogrammed as pluripotent stem cells by demethylation of
the promoter of OCT4 and Nanog through AID , Tet1 and Tet2 pathway.

F-2207

RATIONALLY DESIGNED CELLULAR REPROGRAMMING AND DIRECT CONVERSION

Alex H. Lang[1], Hu Li[3], Darrell N. Kotton[4], James J. Collins[2], Pankaj Mehta[1]
[A]Physics, [B]Biomedical Engineering, [1]Boston University, Boston, MA, United States, [2]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA, United States, [3]Pulmonary Center, Boston University School of Medicine, Boston, MA, United States

Cellular reprogramming and direct conversion holds enormous therapeutic potential for studying and treating human disease. Over the last several years, it has become possible to reprogram differentiated cells into stem cells as well as directly transdifferentiate cells into other specialized cell types by overexpressing specific transcription factors. Present cellular reprogramming and direct conversion strategies design protocols by selecting transcription factors in an ad hoc fashion through a combination of biological knowledge, intuition, and experimental trial and error. Consequently, conversion tends to be inefficient and incomplete. Furthermore, direct conversion protocols only exist between a few select cell types.
We propose to overcome these limitations by inventing a new theoretical framework to describe cellular identity that leads to predictions of transcription factors to use in reprogramming and direct conversion protocols. Our framework provides a method to turn the common developmental metaphor of a rugged epigenetic landscape (Waddington's Landscape) into a mathematically precise landscape. Specifically, we combine genomic data (Affymetrix Mouse microarrays for 95 cell fates) and histone modification data with a technique from physics to explicitly construct a mathematical representation of the epigenetic landscapes underlying cellular identity. In the model, each cell fate is a dynamic attractor, yet cells can change fate in response to external signals, thus capturing the main biological features of development.
To test our theory, we developed a novel tool for generating sets of genes that are "predictive" of specified cell fates, and we tested this tool's potential for predicting reprogramming transcription factors or determining the cell types of a reprogrammed cell populations. Using publically available microarray databases of gene expression and histone modifications (HM), we created a conditional probability distribution of having a HM given a gene expression level and then applied this to create a binary expression state for differentially expressed genes across cell fates. Within our tool, the ``predictivity'' of a gene, in given a cell fate, is related to the correlation matrix between cell types and the binarized HM profile of the genes in the given cell fate. Furthermore, since expression levels are well-fit by a log-normal distribution, the log-normal z-score naturally defines high and low gene expression levels.
Plotting predictivity vs z-score expression, we were able to correctly predict the set of published transcription factors able to reprogram fibroblasts into iPSCs, hepatocytes, and cardiomyocytes. There is ongoing work to validate the direct conversion candidates. Overall, this work suggests epigenetic landscapes are a powerful paradigm for understanding cellular identity and may be useful for rationally designing reprogramming and direct conversion protocols to novel cell fates.

F-2208

HOMOLOGOUS RECOMBINATION DNA REPAIR GENES PLAY A CRITICAL ROLE IN REPROGRAMMING TO A PLURIPOTENT STATE.

Federico Gonzalez[1], Daniela Georgieva[1], Fabio Vanoli[1], Zhong-Dong Shi[1], Matthias Stadtfeld[2], Thomas Ludwig[3], Maria Jasin[1], Danwei Huangfu[1]

[1]Developmental Biology Program, Sloan-Kettering Institute, New York, NY, United States, [2]Department of Cell Biology - Developmental Genetics Program, Skirball Institute of Biomolecular Medicine, New York, NY, United States, [3]The James Comprehensive Cancer Center, Ohio State University - Wexner Medical Center, Columbus, OH, United States

Induced pluripotent stem (iPS) cells hold great promise for personalized regenerative medicine. However, recent studies show iPS cell lines carry genetic abnormalities, suggesting reprogramming may be mutagenic. Here we show that ectopic expression of the reprogramming factors increases the levels of phosphorylated histone H2AX, one of the earliest cellular responses to DNA double strand breaks (DSBs). Further mechanistic studies uncover a direct role of the homologous recombination (HR) pathway, a pathway essential for error-free repair of DNA DSBs, in reprogramming. This role is independent of the use of integrative or non-integrative methods to introduce reprogramming factors, despite the latter being considered a safer approach that circumvents genetic modifications. Finally, deletion of the tumor suppressor p53 rescues the reprogramming phenotype in HR-deficient cells primarily through restoration of reprogramming-dependent defects in cell proliferation and apoptosis. These novel mechanistic insights have important implications for the design of safer approaches to create iPS cells.

F-2211

IDENTIFICATION OF A GENE NETWORK REGULATING THE REPROGRAMMING KINETICS OF DISTINCT CELL TYPES

David Yao, Chi-Kent Ho, Yi Sun
University of California, Los Angeles, Los Angeles, CA, United States

The utility of the induced pluripotent stem cell (iPSC) system has been well-established, with applications ranging from disease modeling, developmental studies, and potential tissue-specific regenerative therapies. However, details of the molecular mechanisms underlying cellular reprogramming to an embryonic cell-like state, especially changes in transcription networks, are still incomplete. Following the observation that different cell types reprogram at various rates, the transcription profiles of four distinct cell types were evaluated in lieu of their reprogramming efficiency, in order to identify the networks of genes that regulate the kinetics of cellular reprogramming. Keratinocytes (Kerat) and human foreskin fibroblasts (hFF) were categorized as slow-reprogramming iPSCs, and amniotic fluid-derived cells (AFDC) and adipose-derived stem cells (ADSC) were categorized as fast-reprogramming iPSCs, based on their relative rates of reprogramming by retroviral transduction with OCT4, SOX2, KLF4, and c-MYC. Weighted Correlation Network Analysis (WGCNA) comparing the transcription profiles of the slow- and fast-reprogramming cell types identified a distinct cluster of genes that were highly correlated within each cell type, and differentially expressed between the two groups. Alterations in these key transcription networks and hub genes may explain the variations in the rate and efficiency of reprogramming different somatic cell types, and provide a better understanding of the cellular reprogramming pathway.

F-2212

SPECIFYING A DEFINITIVE HEMOGENIC PROGRAM

Carlos-Filipe Pereira, Jiajing Qiu, Betty Chang, Xiaohong Niu, Dmitri Papatsenko, Neil R. Clark, Aya Nomura-Kitabayashi, Jason C. Kovacic, Avi Ma'ayan, Christoph Schaniel, Ihor R. Lemischka, Kateri Moore
Icahn School of Medicine at Mount Sinai, New York, NY, United States

Mouse and human fibroblasts can be reprogrammed to pluripotency or directly converted to unrelated somatic cell fates by combinations of transcription factors (TFs). This raised the question whether Hematopoietic Stem

Cells (HSCs) could be generated directly with TFs. Definitive hematopoiesis emerges during embryogenesis via an endothelial-to-hematopoietic transition. We attempted to recapitulate this process using combinatorial TF approaches in mouse fibroblasts containing a transgenic human CD34-based reporter specific to endothelial and hematopoietic stem/progenitor cells. Starting from a pool of eighteen candidate genes, we used an iterative process of elimination to define the optimal TF combination to induce huCD34-positive colonies. We identify a combination of four transcription factors, Gata2, Gfi1b, cFos, and Etv6 that efficiently activate the reporter and induce endothelial-like precursor cells with the subsequent appearance of hematopoietic cells. The precursor cells express the reporter, Sca1 and Prominin1 and are characterized by global endothelial transcription programs. Emergent hematopoietic cells possess HSC gene expression profiles and cell surface phenotypes. When transduced to human fibroblasts this TF combination induces CD34+CD49f+ cells and a subset that also express Prominin1 and low levels of CD45. This result demonstrates the efficacy of this TF combination to impose human HSC-like phenotypes in fibroblasts.
In summary, we demonstrate that a simple combination of TFs is sufficient to specify a complex, dynamic and multistep developmental program in vitro. Our results support the view that programming HSCs is multistep and underscore the requirement of endothelial-like intermediates for HSCs generation. These findings provide insights into the specification of definitive hemogenesis and a platform for future development of patient-specific stem/progenitor cells as well as more differentiated blood products.

F-2213

THE ROLE OF HISTONE METHYL-TRANSFERASES AND DEMETHYLASES IN SOMATIC CELL REPROGRAMMING

Tamer T. Onder[1], George Q. Daley[2,3,4,5,6]
[1]School of Medicine, Koc University, Istanbul, Turkey, [2]Children's Hospital Boston, Boston, MA, United States, [3]Harvard Medical School, Cambridge, MA, United States, [4]Harvard Stem Cell Institute, Cambridge, MA, United States, [5]Brigham and Women's Hospital, Boston, MA, United States, [6]Dana Farber Cancer Institute, Boston, MA, United States

During cellular reprogramming, remodeling the somatic cell chromatin structures poses a major barrier for the activation of pluripotency-specific gene expression. While there is increasing knowledge about the distinct epigenetic states of cells before and after reprogramming, the role of key proteins that regulate chromatin marks in the reprogramming process remains largely unexplored. To examine the influence of chromatin modifiers on somatic cell reprogramming, we have previously employed a loss-of-function approach to interrogate the role of 22 select genes in DNA and histone methylation pathways and identified Dot1L, the only known H3K79 methyl-transferase, as a potent suppressor of cellular reprogramming. Inhibition of Dot1L either by RNAi or a small molecule inhibitor accelerated reprogramming, significantly increased the yield of induced pluripotent stem cell (iPSC) generation, and substituted for two of the exogenous transcription factors (Klf4 and c-Myc) in the reprogramming cocktail. To determine the role of additional histone methyl-transferases and histone demethylases in reprogramming, we conducted a follow-up shRNA survey and identified H3K36 methylation as a barrier to reprogramming. Knock-down of the demethylases for methyl-H3K36 impairs iPSC generation; conversely, suppression of H3K36 methyl-transferases increases reprogramming efficiency. Co-Inhibition of both H3K79 and H3K36 methyl-transferases result in an additive enhancement in reprogramming, suggesting that these two histone modification pathways act redundantly. Our results demonstrate that modulation of chromatin-modifying enzymes can be exploited to more efficiently generate iPSCs and that stability of the somatic cell-state is controlled by the activity of multiple histone methyl-transferases.

F-2215

USING SMALL MOLECULES FOR DIRECT NEURAL CONVERSION - IN VITRO OPTIMIZED GENERATION AND TRANSPLANTATION

Maria Pereira, Ulrich Pfisterer, Shane Grealish, Malin Parmar
Experimental Medical Science, Medical Faculty, Lund University, Lund, Sweden

Recent findings in cell reprogramming showed the possibility of converting somatic cells into induced neuronal (iN) cells by lentiviral overexpression of specific transcription factors. The involvement of only three transcription factors in this process, Ascl1, Brn2 and Myt1L (ABM) was efficient in converting mouse and human fibroblasts, from embryonic, postnatal as well as adult origin. Furthermore, in our lab, we have shown that other specific transcription factors, when used in combination with ABM are able to give rise to subtype-specific iN cells, as the dopaminergic (hDA-iN).
When improving the protocol for iN conversion we found that a delay in the transgenes activation leads to increased conversion efficiency. Also, we sought to determine the possible effect that small molecules have on the generation of converted cells *in vitro* and *in vivo*. Our studies revealed that a delay in the transgenes activation and the use of small molecules, SB-431542, Noggin, and CHIR 99021, highly improves the conversion efficiency and the purity of the generated cells, when compared with previous protocols.
To determine the *in vivo* survival ability of the resulting hiN cells, they were transplanted intrastriatally in adult rats kept under ciclosporin immunosuppression. The conversion genes and DA fate determinants were activated *in vitro* before transplantation, and the cells grafted four and nine days after activation of conversion genes. hiN cells from both transplantation time points showed good survival when analysed four weeks after grafting and hNCAM- and TH-positive cells, generated from the human fibroblasts, could be visualized in the brains of transplanted animals.
This data supports an improvement of iN conversion in comparison to protocols used in previous studies, and show that the addition of small molecules during conversion results in hiN cells that are able to survive after transplantation *in vivo.*

F-2216

HERPES SIMPLEX-1 AMPLICONS: A NON-INTEGRATING, HIGH TRANSGENE CAPACITY VECTOR FOR THE GENERATION OF INDUCED PLURIPOTENT STEM CELLS.

Anna R. Popplestone[1], Michele M. Lufino[1], Sally A. Cowley[2], William S. James[1], Richard Wade-Martins[1]
[A]Physiology, Anatomy and Genetics, [B]William Dunn school of pathology, [1]University of Oxford, Oxford, United Kingdom

Induced pluripotent stem (iPS) cells are a valuable tool for generating patient-specific disease models. Many reprogramming systems have been described since the first report by Yamanaka in 2006 and iPS reprogramming has been achieved using a variety of methods such as lentivirus, retrovirus, plasmids, small molecules, RNAi and transposons in various cell types. However, some technological concerns still remain, including insertional mutagenesis caused by integrating viruses and low efficiency for the non-integrating alternatives.
Herpes simplex virus type-1 (HSV-1) amplicons are replication-deficient vectors with a large transgene capacity of 150 kb. HSV-1 amplicons address the main limitations of current reprogramming methods as they are non-integrating and have high transduction efficiency in a variety of cell types. We have developed HSV-1 amplicon reprogramming vectors which have several unique features including: the Epstein Barr Nuclear Antigen 1 (EBNA-1)/oriP cassette for episomal retention and replication of the vector; the presence of multiple reprogramming genes driven by a single promoter; and, the thymidine kinase (TK) suicide gene for efficient removal of retained vectors following reprogramming.
Vector constructs were characterised to ensure all elements are functional. Western blot analysis of transfected cells confirmed expression of the reprogramming factors Sox-2, Oct-3/4, Klf-4 and c-Myc, and DNA extracted from transfected clonal cells lines confirmed the extra-chromosomal retention of the vector. Functionality of the TK cassette was verified by a ganciclovir sensitivity assay.
Initial attempts to package the vector yielded lower than expected amplicon titres which we hypothesised was due to the expression of reprogramming genes during packaging. The pCMV promoter was replaced with a pCMVTetO2 promoter which can be silenced during packaging by co-transfection of a Tetracycline repressor encoding plasmid. This restored the vector titre to normal whilst still maintaining constitutive expression of the transgenes in absence of the repressor plasmid.
Initial reprogramming experiments in human fibroblasts resulted in partially reprogrammed colonies. Colony structures were observed 12 days after transduction, however they did not have typical iPS morphology. We hypothes-

ised that extra factors may be needed for complete reprogramming to occur. Studies based on other EBNA-1 based systems require supplementary reprogramming factors Lin-28 and Nanog and recent literature suggest that a higher Oct4 ratio of three times is optimal for successful reprogramming.
The first vector was therefore modified to carry a second copy of Oct4 and an additional HSV-1 amplicon vector was constructed carrying the factors Sox-2, Oct-4, Lin-28 and Nanog. Together these vectors provide an optimal relative factor expression of 3x Oct-4, 2x Sox-2 and 1x (Klf-4, C-Myc, Lin-28 and Nanog). Preliminary reprogramming experiments using co-transductions of these amplicons yielded GFP positive colonies which will be further characterised as potential iPS colonies.
In conclusion, the HSV-1 amplicon vectors provide a non-integrating, episomally retained reprogramming system capable of high transduction efficiency. This addresses issues with current methods and the large transgene capacity allows delivery of multiple reprogramming factors.

F-2217

SOMATIC CELLS ARE PREDISPOSED TO THEIR RESPONSE AND FATE DURING IPSC REPROGRAMMING

Roni Rosner[1], Inbar Pilzer[1], Zachary D. Smith[2], Alexander Meissner[2], **Iftach Nachman**[1]
[1]Biochemistry and Molecular Biology, Tel Aviv University, Tel Aviv, Israel, [2]Stem Cells and Regenerative Biology, Harvard University, Cambridge, MA, United States

Reprogramming to pluripotency typically results in a low success rate (~1% or less), with other cells showing anything between partial reprogramming to no response. It is not clear when cells undertake a decision to reprogram, to what extent the process is stochastic or if certain cellular states provide predispositions towards a given fate early in the timeline. It is equally unclear whether the activation of pluripotency and self-renewal depend on a single decision or on a series of sequential decisions. Recent work revealed large cell-to-cell variability in gene expression at early phases of the process, potentially suggesting stochastic alterations during these early phases.
Here we use live cell imaging of the reprogramming process to dissect the timing of fate decisions during the first two weeks of ectopic factor induction using clonal 2ndary mouse embryonic fibroblasts. Using multiple color tagging, we trace cell lineages from the divisions that precede the induction of reprogramming factors through to the generation of iPSC colonies. This strategy allows us to define lineage pairs, with each pair emanating from a pre-induction cell division and founder. We find that the response to the induction of reprogramming factors, and ultimately to the generation of iPSCs, is similar within lineage pairs. Using this data, we provide statistical evidence that any major fate decision can be predicted within the founding cell, prior to the division that creates the lineage pair, as well as before factor induction. Using spatial correlation analysis we rule out an alternative explanation to these results based on local niche effects. We further show that cells of origin that contribute to partial or fully reprogrammed lineages differ from non-responding cells in their pre-induction proliferation rates, supporting the idea that differences in internal state between uninduced cells can contribute to how they respond. To check whether different lineage responses stem from differential OKSM expression, we analyze the correlation of morphological response with factor levels at early timepoints and show that the initial cellular response, as reflected by nuclear size, does not depend on the level of any of the trans factors.
Our results suggest that cells within the reprogramming population are predisposed towards different responses to ectopic factor induction, including growth arrest, transformation/propagation as low stringency intermediates, or formation of iPSC colonies within a standard reprogramming timeline. In light of this, we interpret recent results as highlighting pre-existing cell-to-cell epigenetic variability within the population at the time of factor induction. Exploring this variability may provide a refined model for reprogramming that includes the epigenetic status of the founding cell, and may benefit efforts to characterize or predict responses to ectopic transcription factors as they drive changes in cellular fate.

F-2218

MODULATING GROWTH FACTOR ADDITION TO ESSENTIAL 6^{TM} MEDIA FOR A COMPLETE XENO-FREE MEDIA FROM FIBROBLAST CULTURE TO IPSC GENERATION AND EXPANSION

Alexandria Sams[1], Shayne Boucher[1], Rene Quintanilla[2], Andrew Fontes[2], Uma Lakshmipathy[2]
[1]Primary and Stem Cell Systems, Life Technologies, Frederick, MD, United States, [2]Primary and Stem Cell Systems, Life Technologies, Carlsbad, CA, United States

The generation of induced pluripotent stem cells (iPSCs) from somatic cells has the potential to revolutionize cell-based therapeutic applications. Current methods require multiple media systems for various stages of reprogramming. An ideal solution would be a media system that can be used from the original patient sample harvest to expansion of the resulting iPSC clones.
Essential 8™ media has emerged as an easy, simple and cost effective media system for the expansion of pluripotent ESC and iPSCs. Here, we use the basal version without growth factors, termed Essential 6™ media as the base media and optimize the addition of the two key growth factors, bFGF and TGFβ, at various stages during reprogramming. Our results suggest that unlike other xeno-free media systems, Essential 8™ media supports efficient transduction via Sendai virus without toxicity. Furthermore, optimal timing of TGFβ removal a week after transduction supported efficient reprogramming resulting in iPSC colonies that were subsequently expanded on recombinant human vitronectin and Essential 8™ media. Characterization studies indicated a normal karyotype, pluripotency marker expression, and in vitro differentiation into all three lineages after multiple passages. While use of the Essential 8™ media system has been reported for episomal reprogramming, its optimization in other reprogramming methods and workflows enables a modular, easy to use, feeder-free, xeno-free system.

F-2221

CELL FATE MANIPULATION WITH SYNTHETIC MRNA

Johanna E. Goldmann[1], Brad Hamilton[2], Rudolf Jaenisch[3]
[1]Whitehead Institute, Cambridge, MA, United States, [2]Stemgent, Cambridge, MA, United States, [3]Biology, Whitehead Institute and MIT, Cambridge, MA, United States

Recent advances in our ability to manipulate cell fate choices and to direct cellular fate through reprogramming and transdifferentiation have revolutionized both the field of basic developmental biology as well as that of regenerative medicine.
These fields have made great strides but face the remaining challenge that most current approaches to cell fate manipulation require permanent genome alteration and offer limited control of temporal and stoichiometric delivery of factors required to manipulate cell fate. Reprogramming with synthetic mRNA provides an effective and non-integrating strategy for cell fate manipulation as well as offering control over stoichiometry and kinetics. However, previous approaches of mRNA-based reprogramming were laborious, requiring a total of 17 transfections and results often not reproducible. Here we report several significant improvements to the existing methods that increase both the efficiency and the robustness of the method.
The improvements to the current method establish mRNA mediated reprogramming as one of the most efficient and reliable methods to reprogramming somatic cells to the pluripotent state.
By adopting a feeder free approach to reprogramming and incorporating miRNA, we were able to reduce the number of transfections required to generate iPS cell colonies to 10, with colonies that could be isolated from the primary culture as early as at day 12. Further, by controlling for both stoichiometry and kinetics of the reprogramming factors, the reprogramming process was significantly enhanced.
Addition of these changes to existing mRNA reprogramming protocols resulted in the efficient derivation of iPS cell colonies from both BJ fibroblasts as well as primary adult patient fibroblasts such that we were able to effectively reduce the number of required transfections from 10 to 6, with colonies appearing as early as day 5. Furthermore the decreased number of transfections did not occur at the cost of reprogramming efficiency but instead increased it by more than two-fold. The iPSC colonies isolated from both the BJ and primary patient fibroblasts were isolated after 7-10 days, are stable and express pluripotency makers (SOX2, OCT4, NANOG) are karyotypically normal and can generate teratomas.

F-2222

REPROGRAMMING NON INCORPORATING EMBRYONIC STEM CELLS INTO A FUNCTIONAL STATE

Ricardo A. Rossello[1], Andreas Pfenning[2], Ute Hochgeschwender[3]

[1]Biochemistry, University of Puerto Rico, Medical Sciences Campus / The University of Puerto Rico Comprehensive Cancer Center, San Juan, PR, United States, [2]Computational Biology, MIT, Cambridge, MA, United States, [3]Neurobiology, Duke University, USA, NC, United States

Human embryonic stem cells are said to be in a primed state. Primed pluripotent cells, can give rise to differentiated teratomas, but are highly inefficient in repopulating the ICM upon aggregation or injection into host blastocysts. Cell reprogramming is a powerful tool that may be used to address a plethora of issues in biology ranging from understanding evolution, to studying and treating diseases. As such, a cell reprogramming strategy may be used to test for recapitulate a potential human naïve stem cell state.

As a model for such a concept, mouse ES cells can be utilized. Derivation of mouse embryonic stem cells (ESCs) is a lengthy process that sometimes produces cell lines that have all of the features inherent in this cell type, but fail to incorporate into the germline. Identifying this limitation may take up to several months, while the cell line is established. This limiting obstacle may provide a template for human primed cells, and therefore a case to study if the functional “naïve” state could re-activated.

Here, we determine the differential expression patterns in 5 pairs of mouse cell lines derived from different strains (5 functional and 5 non incorporating) and test the hypothesis that ESC functionality can be restored. Each pair of functional and non-incorporating cell line was generated from a single mouse. Using microarray and bioinformatic analysis, we determined a priority list of differentially expressed genes. The list included genes such as HBEGF, CCR5, ATRX, RNF17, TCF712, Tex9, KLF-5 USP 48 and ERAS, which were significantly downregulated in the non incorporating ESCs; the expression level of these genes was also validated using qRTPCR. CDNAs for these genes were isolated and used to construct gene cassettes and lentiviral vectors. Non incorporating cells were induced to overexpress these genes. Expression levels were measured, and the cell lines were reprogrammed for 9 days. Here we report that, at least one combination of transfections (ERAS+ATRX+RNF17) in non-incorporating cells was able to alter the expression profile and establish functionality. This was determined by in-vivo chimeric generation of mouse embryos (4 day old) with reprogrammed non incorporating cells. Reporgrammed cells showed incorporation, whereas their non reprogrammed counterparts did not. In addition, teratoma formation exhibited full healthy tissue formation in the reprogrammed state where as some limitations in tissue formation and organization were observed in the non reprogramed control. It is important to note that iPS lines generated from these mice were also generated as a positive control. They were used throughout the experiments and incorporated. However, when the non-incorporating cells were treated with the Yamanaka factors, incorporation was not achieved.

This suggests that, through reprogramming, it is possible to restore the functional naïve state of a non incorporating mESC. The results may be a translational gateway into reprogramming primed Human ESCs, into a naïve state with full ESC features and function.

F-2223

NON-VIRAL DIRECT NEURONAL REPROGRAMMING: SERIAL TRANSFECTION OF MOUSE FIBROBLASTS WITH BIOREDUCIBLE POLYMER-DNA NANOCOMPLEXES DRIVES EFFICIENT CONVERSION TO FUNCTIONAL NEURONAL CELLS

Andrew F. Adler[1], Christopher L. Grigsby[1], Karina Kulangara[1], Hong Wang[2], Russell C. Addis[3], Ryohei Yasuda[2], Kam W. Leong[1]

[A]Biomedical Engineering, [B]Neurobiology, [1]Duke University, Durham, NC, United States, [2]Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA, United States

Direct neuronal reprogramming, where differentiated cells are converted into neurons without proceeding through an intermediate proliferative (and potentially teratogenic) pluripotent stem cell-like state, is a promising new tool in the arsenal against neurodegenerative disease. Wernig et al. first described the direct reprogramming of fibro-

blasts into functional induced neuronal cells (iNs) utilizing ectopic expression of a neuronal cocktail of transcription factors (TFs). This breakthrough technique has since been extended to the production of many clinically relevant neuronal subtypes, but to date these systems universally rely on retroviruses for the efficient delivery of TF genes, miRNAs, or shRNAs for direct neuronal reprogramming. iNs produced with viral transduction are invaluable for disease modeling, drug discovery, and the fundamental study of direct reprogramming, but their clinical translatability is hindered by concerns of genotoxic integration. As such, we have developed a non-viral system for the direct reprogramming of mouse embryonic fibroblasts into electrophysiologically active neuronal cells. We employed serial transfections of exceptionally nontoxic bioreducible p(CBA-ABOL) (poly cystaminebisacrylamide-4-amino-1-butanol) polyplexes containing plasmids encoding the neuronal TFs Brn2, Ascl1, and Myt1l (BAM factors) or Ascl1, Lmx1b, and Nurr1 (ALN factors) for the production of iNs resembling excitatory cortical or dopaminergic neurons, respectively. Repeated p(CBA-ABOL) transfections of BAM factors produced a nonlinear improvement in the yield of Tuj1+ non-virally induced neuronal cells (NiNs), up to a 7.6% conversion efficiency for five serial doses, without the compounding toxicity incurred through repeated use of Lipofectamine 2000. NiNs produced with the BAM factors expressed the pan-neuronal cytoskeletal and synaptic proteins MAP2, Tuj1, tau, and synaptophysin, and those produced with the ALN factors expressed tyrosine hydroxylase - the rate-limiting enzyme in the biosynthesis of dopamine. To identify neuronal cells for electrophysiological recordings, BAM factor NiNs were transduced with a lentiviral reporter driving expression of RFP under control of the neuron-specific synapsin I promoter. Over 90 percent of patch-clamped synapsin-RFP+ cells fired action potentials upon injection of depolarizing current. These results demonstrate the feasibility of direct non-viral neuronal reprogramming, and may be amenable to other therapeutic transdifferentiation targets. Our ongoing work aims to reprogram human cell sources, to deepen our functional characterization of NiNs, and to further improve the yield and homogeneity of NiN generation through the use of epigenetic modifiers and polycistronic vectors.

F-2224

A COMPARISON OF REPROGRAMMING METHODS

Thorsten M. Schlaeger[1], Laurence Daheron[2], Maureen S. Lynes[2], Thomas Brickler[2], Karrie Chan[3], Amelia Cianci[1], Alexander DeVine[1], Samuel Entwisle[2], Andrew Ettenger[1], Kelly Fitzgerald[1], Michelle Godfrey[3], Dipti Gupta[3], Jade McPherson[3], Alexander Meissner[2], Kelly Fitzgerald[3], Chad A. Cowan[2], Lee L. Rubin[2], George Q. Daley[1]
[1]Stem Cell Program, Boston Children's Hospital, Boston, MA, United States, [2]HSCI, Cambridge, MA, United States, [3]Children's Hospital Boston, Boston, MA, United States

The first human induced pluripotent stem cells (hiPSCs) were derived using retro/lentiviral gene transfer methods that permanently alter the genome and often leave residual transgene expression or reactivate expression during in vitro differentiation. A number of alternative techniques for generating integration-free hiPSC lines have since been developed. Transduction with Sendai viral particles (SeV; Fusaki et al. (PMID: 19838014), Invitrogen), nucleofection of EBNA1/OriP-based replicating episomal plasmids (Okita et al. (PMID: 21460823), Addgene), and serial lipofection of synthetic mRNAs (Warren et al. (PMID: 20888316), Stemgent) are efficient integration-free gene delivery approaches for human somatic cell reprogramming, but a relative assessment of their advantages and limitations has not yet been reported. We have performed a comprehensive cross-comparison of these three non-integrating reprogramming techniques and conventional retro- and lentiviral methods based on a number of criteria including reprogramming efficiency, reliability, speed of colony emergence, kinetics of loss of exogenous factors, cost, ease of use, and quality and integrity of the resulting lines. Reprogramming experiments were performed in parallel in two separate laboratories to account for inter-lab variability. At least three iPS cell lines were generated per method for three somatic cell sources including two spinal muscular atrophy (SMA) patient derived skin fibroblast samples. Parental somatic cells and human embryonic stem cell samples were processed in parallel as controls. To assess hiPSC quality we compared the samples by protein marker and gene expression analysis, in vitro differentiation (Scorecard assay, Bock et al., PMID: 21295703), karyotyping, and copy number variation analysis. In this presentation we will summarize and discuss the specific advantages and drawbacks of each method. Based on our overall assessment, we chose to adapt the episomal reprogramming technique into a current Good Manufacturing

Practices (cGMP) conforming protocol for integration-free reprogramming of primary skin fibroblast and peripheral blood samples.

F-2225

IN VIVO DEDIFFERENTIATION IN A NEW REPROGRAMMABLE MOUSE

Maria Abad[1], LLuc Mosteiro[1], Cristina Pantoja[1], Sagrario Ortega[2], Marta Cañamero[3], Orlando Domínguez[4], Teresa Rayón[5], Inmaculada Ors[5], Miguel Manzanares[5], Manuel Serrano[1]
[A]Molecular Oncology, [B]Transgenic Mice Unit, [C]Histopathology Unit, [D]Genomics Unit, [1]CNIO, Madrid, Spain, [2]Cardiovascular Develpment and Repair, CNIC, Madrid, Spain

The ability to reprogram differentiated cells into induced pluripotent stem cells (iPSCs) has considerably improved our current understanding of cellular plasticity and has also helped to paving the way towards regenerative medicine. However, little is known about whether or not in vivo reprogramming is feasible and if so, what type of cells are generated in vivo and what are their implications in the organism. We have generated a “reprogrammable” transgenic mouse strain that ubiquitously express the so-called Yamanaka factors upon treatment with doxycycline. I will present data that we have obtained by using this mouse model regarding the effects of the in vivo over-expression of the reprogramming factors. Our results are likely to impact on the development of future applications both in regenerative and in reproductive medicine.

F-2226

STRATEGY FOR PROTEIN INDUCED REPROGRAMMING USING 30Kc19 PROTEIN CONJUGATED TRANSCRIPTION FACTORS

Jina Ryu[1], Hee Ho Park[2], Hong Jai Lee[2], Jongmin Lee[2], Tai Hyun Park[2]
[A]Interdisciplinary Program, Bioengineering, [B]School of Chemical and Biological Engineering, [1]Seoul National University, Seoul, Korea, Republic of

Recently, 30Kc19 protein from *Bombyx mori* was found to have several biological advantages, including enzyme-stabilizing effect and cell-penetrating property. Particularly, the cell-penetrating property made it for the delivery of conjugated cargo protein with the 30Kc19 protein, without cell specificity. Hence, we applied these properties of 30Kc19 protein on cellular reprogramming. Protein-based approach is an alternative method to the virus delivery system, which has a problem of host genomic integration. Few years ago, protein-induced pluripotent stem cells (p-iPSCs) were carried out by other research groups, but low efficiency on reprogramming was a bottleneck for p-iPSCs research. In our study, each transcription factor (Oct4, Sox2, c-Myc, Klf4) was cloned with 30Kc19 in pET23a vector and each was expressed in *E. coli* with optimal culture conditions for protein production. Normally, all four transcription factors are produced as inclusion bodies and require additional refolding process, however, by conjugating to 30kc19, the purified Oct4, Sox2, Klf4 proteins were produced as soluble forms and they all had cell-penetrating property. Also, the stability of the 30Kc19-conjugated transcription factors was higher at 37℃ that of the 9R-conjugated transcription factors. Especially, Oct4-30Kc19 and Sox2-30Kc19 proteins maintained their structures by more than 70%, whereas Klf4-9R maintained its structure by less than 10% in 12 h incubation at 37℃. Furthermore, *in vivo* assay showed that our 30Kc19-conjugated transcription factors had very low cellular toxicity even with high concentration (60 μg/ml of total protein). Bearing these advantages in mind, we optimized the protein transduction process, such as protein concentration, time and frequency. To confirm the transduction, combination of one protein and three viruses will be tested for reprogramming. In conclusion, this strategy is anticipated to be used in optimizing the protein-based cellular reprogramming.

F-2227

PARTIALLY REPROGRAMMED RAT VASCULAR SMOOTH MUSCLE CELL STIMULATED CORONARY COLLATERAL GROWTH IN A RAT REPETITIVE ISCHEMIA MODEL

Liya Yin, Vahagn Ohanyan, Molly Enrick, Kelly Stevanov, Christopher Kolz, Suzanna Logan, Angelo Delucia, William Chilian
Integrated Medicine Science, Northeast Ohio Medical University, Rootstown, OH, United States

Although coronary heart diseases (CHD) are the leading cause of mortality and morbidity in the United States, not all patients with CHD have severe manifestations of the disease. The extent of the coronary collateral circulation has a large ameliorating influence, and reduces the incidence of sudden death and also the size of the infarct following a coronary occlusion. Although the importance of collateral growth is well recognized, attempts to therapeutically stimulate this growth have largely failed. Because growing coronary collateral vessels as "natural bypasses" would serve as a prophylactic procedure to prevent myocardial infarction and sudden death, we believe that coronary vascular growth may be an important target in regenerative medicine. Therefore, we studied stem cell therapy directed to the restoration of coronary blood flow by stimulation the growth of coronary collateral vessels. To address this problem, we sought to develop, via reprogramming, a progenitor type cell, that would stimulate collateral growth in the heart, without concerns of pluripotency that could result in undesirable cell types. To accomplish this, we partially reprogrammed rat vascular smooth muscle cells (VSMCs) into vascular progenitor cells with doxycycline inducible lentivial system expressing Oct4, Sox2, Klf4 and c-Myc. Partially reprogrammed VSMCs (pr-VSMC) behaved similarly to the inducible vascular progenitor cells (iVPCs) reprogrammed from rat vascular endothelial cells that we reported previously. pr-VSMC expressed stem cell markers SSEA-1, Oct4 and progenitor cell markers CD133, Flk-1 and c-kit (FACS analysis). The DNA methylation of Oct4 and Nanog promoters in the partially reprogrammed VSMCs was higher than fully reprogrammed iPS cells measured by bisulfate genomic sequencing analysis. Interestingly, in a 2-dimensional cell culture system, the pr-VSMCs appeared to form tube-like structures and possible lumen formation. Importantly, the pr-VSMCs did not form teratomas when injected into the capsule of kidney of FOXC-SCID mice, suggesting they were not pluoripotent. To evaluate whether pr-VSMCs would stimulate coronary collateral growth, we used them in a rat model of repetitive ischemia (RI), which produce the brief (40 sec) occlusions by a pneumatic occluder and do not produce an infarct, but stimulate collateral growth. Cardiac function was measured by echocardiography before and after inflation of the occluder (we compared the decrease in function during the occlusion_if collateral flow is adequate to maintain function, there should not be a decrease during occlusion). Collateral flow was measured during inflation of the occluder with microspheres and expressed as a ratio (microsphere counts [dpm/g] in the tissues) between the collateral-dependent zone and normal zone (CZ/NZ). Ideally, the ratio of CZ/NZ should be 1, if collateral flow equals that to the normal zone. The results show that, compared to the RI control rat without cell injection, the partially reprogrammed VSMCs augmented coronary collateral growth by increasing the CZ/NZ ratio (0.48 vs 0.7237). The decrement in cardiac function during occlusion was significantly improved in the group treated with the pr-VSMCs. The percentage change of left ventricle EF and FS during inflation of the occluder in pr-VSMCs treated group decreased from (10.99%, 10.46%) before RI to (1.21%, 1.58%) after RI ($p<0.05$). These results suggest that pr-VSMCs stimulated coronary collateral growth in vivo.

F-2228

JARID2 IMPROVES THE KINETICS AND EFFICIENCY OF TRANSCRIPTION FACTOR-BASED REPROGRAMMING

Hiroyoshi Iseki[1], Yutaka Nakachi[1], Tomoaki Hishida[1], Yoko Tanimoto[2], Fumihiro Sugiyama[2], Ken-ichi Yagami[2], Akihiko Okuda[1], Yasushi Okazaki[1]
[1]Research Center for Genomic Medicine, Saitama Medical University, Hidaka, Japan, [2]Laboratory Animal Resource Center, University of Tsukuba, Tsukuba, Japan

Embryonic stem cells (ESCs) and oocytes contain factors capable of nuclear reprogramming of somatic cells. In recent reports, reprogramming of somatic cells to a pluripotent state was achieved by four transcription factors (Oct3/4, Sox2, Klf4 and c-Myc; OSKcM) which are essential to the ESC identity. However, the generation of induced pluripotent stem cells (iPSCs) by OSKcM was shown to be lower efficiency and slower kinetics than that of the cell

fusion- and the nuclear transfer-based reprogramming, suggesting that any additional factors are required for efficient and proper reprogramming. Indeed, several works demonstrated that iPSC generation was facilitated by additional factors with OSKcM. To identify genes promoting iPSC generation, we have examined genes exhibiting relatively high expression in both ESCs and oocytes. Here we report that Jarid2, an ESC/oocyte factor, improves mouse and human iPSC generation. Forced expression of Jarid2 in mouse embryonic fibroblasts (MEFs), mouse tail-tip fibroblasts, and human dermal fibroblasts increased the number of iPSC colonies induced by OSKcM. Using a set of Jarid2 deletion mutants, we identified that the N-terminal half of Jarid2 (Jarid2ΔC) was sufficient to enhance mouse and human iPSC generation. Since Jarid2 could promote iPSC generation more efficiently when c-Myc was present, we constructed retrovirus vectors that co-express Jarid2 and c-Myc using 2A peptide system. The iPSC generation using a co-expression vector clearly showed improvement of kinetics and efficiency of reprogramming by Jarid2ΔC. We next examined the characteristics of mouse iPSC clones generated from MEFs by retroviral transduction of OSKcM with Jarid2 (Jarid2-miPSC). Jarid2-miPSC clones differentiated into all three germ layers and contributed to generating chimeric mice. Interestingly, retroviral transgene silencing, a hallmark of completely reprogrammed iPSCs, was observed in all of six Jarid2-miPSC clones, but not in four out of six empty control-miPSC clones. The transgene expressions in empty-miPSC were silenced after six additional passages. These findings strongly demonstrate that Jarid2 enhances iPSC generation and that Jarid2 may improve the quality of iPSCs at the epigenetic level. Finally, we evaluated the effect of Jarid2 knockdown on iPSC generation. Knocking down Jarid2 in MEFs impaired iPSC generation, which was rescued by ectopic expression of Mdm2 and a dominant negative form of p53. Furthermore, RT-qPCR revealed that Jarid2 knockdown up-regulated the expression of senescence markers *p15* and *p19ARF*, whereas Jarid2 overexpression suppressed these expressions. These results suggest that the promotion of iPSC generation by Jarid2 is associated with the p19ARF-Mdm2-p53 pathway.

F-2231

RNA MEDIATED GENERATION OF INTEGRATION-FREE IPS CELL LINES FROM HUMAN BLOOD DERIVED CELL TYPES

Sarah Eminli, Rebekah Ashley, Brad Hamilton
Research and Development, Stemgent, Cambridge, MA, United States

In 2010, it was demonstrated that efficient repeated transfection of fibroblasts with a cocktail of reprogramming associated mRNAs resulted in the efficient derivation of integration-free human iPS cells. Subsequent enhancements including the incorporation of a reprogramming associated miRNA cocktail, the addition of a highly efficient and proprietary RNA transfection reagent, and the inclusion of Matrigel and other defined ECMs resulted in a simplified xeno-free protocol that reduced the number RNA transfections and ultimately the number of days required to derive human iPS cell lines from adult patient specific fibroblasts to 10-12 days. To date, no group has been able to demonstrate effective reprogramming of a blood derived cell type with non-integrating RNAs. Here we present novel data demonstrating efficient delivery of RNAs into various cell types from human blood for the generation of integration-free iPS cells.
Peripheral blood provides easy access to adult human cell types for reprogramming purposes. Likewise, umbilical cord blood is routinely collected and cryopreserved and available for this purpose as well. As a result there are numerous studies utilizing these frozen patient cell samples from existing blood banks to generate patient and disease specific iPS cell lines for regenerative medicine applications and disease study. Additionally the ability to derive integration-free iPS cells from human blood with mRNA enables the study of myeloproliferative disorders in which the disease-causing somatic mutations are restricted to blood cells and therefore inaccessible in alternative target cell types for reprogramming purposes.
While human blood derived cell types are easily accessible, common lipid-based transfection reagents are notably inefficient at delivering both small and large RNA species to these types of cells. The Stemfect® RNA Transfection Kit was developed for and has demonstrated efficient and non-toxic delivery of RNAs to a variety of common cell types including human ES and iPS cells. The application of this novel RNA delivery system to reprogram blood derived cell types has resulted in the highly efficient delivery of mRNA (> 80%) to both suspension blood cells and adherent endothelial progenitor cells derived from human peripheral blood. Preliminary data suggests that repeated trans-

fection of these blood derived cell types with reprogramming associated RNAs results in morphological changes indicative of pluripotency.

F-2232

GENERATION OF OVINE IPS CELLS WITH DEFINED FACTORS

Lei Bao[1], Lixiazi He[1], Lei Xiao[2]

[1]Shanghai Inst of Biochemistry & Cell Biology, Shanghai, China, [2]Zhejiang University, Hangzhou, China

Sheep have been used to study somatic cell reprogramming for a long time since Dolly was generated by somatic cell nuclear transfer (SCNT). Gene modification in sheep to improve their production traits, disease resistance ability, and ability to produce valuable, high-quality proteins has been widely applied in agriculture and biomedicine. However, sheep embryonic stem (ES) cells are still not available, which limits the generation of precise gene-modified sheep. In this study, we found that sheep somatic cells can be directly reprogrammed to induced pluripotent stem (iPS) cells using defined factors (Oct4, Sox2, c-Myc, Klf4, Nanog, Lin28, SV40 large T and hTERT). Our observations indicated that somatic cells from sheep are more difficult to reprogram than somatic cells from other species, in which iPS cells have been reported. We demonstrated that sheep iPS cells express ES cell markers, including alkaline phosphatase, Oct4, Nanog, Sox2, Rex1, stage-specific embryonic antigen-1, TRA-1-60, TRA-1-81 and E-cadherin. Sheep iPS cells exhibited normal karyotypes and were able to differentiate into all three germ layers both in vitro and in teratomas. Our study may help to reveal the mechanism of somatic cell reprogramming in sheep and provide a platform to explore the culture conditions for sheep ES cells. Moreover, sheep iPS cells may be directly used to generate precise gene-modified sheep.

F-2233

COMBINATION OF SYNTHETIC MRNA AND MIRNA ENABLES HIGHLY EFFICIENT REPROGRAMMING OF RECALCITRANT CELL TYPES FOR A WIDE RANGE OF DISEASE MODELS

Sina Atashpaz[1], Pietro Lo Riso[1], Antonio Adamo[1], Giuseppe D'Agostino[1], Pierre-Luc Germain[1], Paolo Prontera[2], Nicoletta Colombo[1], Ugo Cavallaro[1], Giuseppe Merla[3], James Kehler[4], Brad Hamilton[4], Bryan Roberts[4], Giuseppe Testa[1]

[1]European Institute of Oncology, Milan, Italy, [2]Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Perugia, Italy, [3]Medical Genetics Unit, Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Italy, [4]Stemgent, Cambridge, MA, United States

The generation of induced pluripotent stem cells (iPSC) from terminally differentiated cells holds great promise for disease modeling, drug discovery and regenerative medicine. Ectopic expression of defined pluripotency factors generates patient-specific pluripotent cells that can be then differentiated into relevant cell types to gain mechanistic insights into disease physiopathology. However, iPSC-based modeling is limited by: i) low efficiency of iPSC derivation; ii) the risk of insertional mutagenesis and iii) residual transgene expression from integrated vectors. To tackle these issues, many non-integrating platforms have been developed. Warren et al. first described the ability to reprogram human cells using modified mRNA with conversion efficiencies and kinetics superior to DNA-based methods. Various reports also defined the ability of specific clusters of miRNA to reprogram somatic cells in the absence of exogenous reprogramming transcription factors. While the work of Anokye-Danso et al. relied on integrating lentiviral vectors to express miRNA clusters for the efficient derivation of iPSC, Miyoshi et al. were able to deliver synthetic miRNA to successfully reprogram somatic cells, albeit at a low efficiency.
Here for the first time, we unveil the combinatorial potential of the mRNA and miRNA approaches by presenting two clinically relevant examples: i) the generation and full characterization of iPSC from a large cohort of patients affected by Williams-Beuren Syndrome and Autism Spectrum Disorders based on miRNA enhanced-mRNA reprogramming in feeder-free condition; and ii) the efficient generation of integration-free iPSC from both high grade and low grade ovarian cancer (OC) samples, establishing an innovative approach to model tumor pathogenesis.

In both cases, we validated pluripotency on the basis of expression of well-recognized pluripotency markers, maintenance of undifferentiated morphology upon several passages in feeder-free conditions and tri-lineage and lineage-directed differentiation in vitro and in vivo. Moreover, we assessed genomic integrity at high resolution, finding only few CNV, the overwhelming majority of which pre-existed in parental cells, consistent with recent studies showing CNV mosaicism within parental cells prior to reprogramming.
Our results show that the combination of miRNA and mRNA based approaches through a novel RNA delivery technology, besides preventing insertional mutagenesis and the persistent expression of reprogramming factors inherent to viral-based technologies, offeris unprecedented efficiency and succeeds in reprogramming also refractory samples (as in the case of fibroblasts from elderly individuals or OC samples that had resisted traditional reprogramming). Furthermore, the addition of proprietary miRNAs to the previously described mRNA cocktail (OCT4, SOX2, KLF4, c-MYC, LIN28) further shortens the process to less than two weeks, reducing the time needed to obtain clinically relevant integration-free human iPSC and without detectable accumulation of reprogramming-induced mutations.

F-2234

TURNING SKIN INTO CARTILAGE: A TRANSDIFFERENTIATION STRATEGY FOR CARTILAGE REGENERATION

Perla A. Cota-Elizondo, Mehdi Shafa, Shiying Liu, Derrick E. Rancourt
Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, Canada

Table of Contents

Introduction
Hyaline cartilage degeneration is the biggest cause osteoarthritis (OA). Since cartilage does not regenerate, there is optimism that cartilage may be repaired using a combination of pluripotent stem cells and tissue engineering. Recently great strides have been made in generating autologous pluripotent stem cells via the process of cellular reprogramming, where skin cells are regressed to an embryonic stem cell state. A key step in cellular reprogramming is the opening of chromatin. In newer trans-differentiation schemes, instead of reverting cells all the way to the embryonic state, they can instead be redirected to specific cell types. In this pilot study, we are investigating our ability to generate chondrocytes from mouse fibroblasts using a trans-differentiation strategy.
Methods
Retroviral vectors with the 4 reprogramming factors (Oct4, Sox2, Klf4 and c-Myc), were packaged with a Plat-E cell line. Next the viral particles were added to mouse embryonic fibroblast (MEF). The following day the cell medium was changed to a reprogramming medium with 15% FBS (first 6 days). After day 6, FBS was reduced to 1% until day 9. JAK-STAT inhibitor (JI1) was added for the first nine days. On day 10 cells were passaged with a chondrocyte differentiation medium. After four weeks, resulting aggregates were assayed for type II collagen (Col 2) via immunofluorescence or for chondrocyte and osteoblast markers using RT-PCR. Samples were also taken at six weeks, and injected in the femoral muscle of SCID mice. Three weeks post injection; the formed tissues were isolated and dissected for their tissue assessment by H&E staining.
Results
The first insight of transient reprogrammed MEFs was their immediate morphology change. This suggested that cells had begun the reprogramming process. They did not form induced pluripotent stem cell colonies, because of the absence of leukemia inhibitor factor (LIF) and the addition of JI1 in the medium for nine days. On day 10 they were changed to a differentiation medium where at week 4, aggregates were taken for immunofluorescence and

RT-PCR assays. The presence of Col2 was clearly observed in the immunostaining assay. Using RT-PCR both chondrocyte and osteoblast markers were found to be represented suggesting that by week 4 chondrocytes had already begun to undergo the process of endochondral ossification *in vitro*. This notion was confirmed by our observation that both cartilage and bone tissue was observed at the site of transplantation.

Conclusions

Our preliminary trans-differentiation results have shown to be a very effective strategy for directly generating cartilage tissue from skin cells. This offers the opportunity to circumvent the iPSC derivation step, thereby avoiding the potential problem of teratoma formation following transplantation. It may also have the added advantage of shortening the timeline for autologous cartilage production, thus facilitating accessibility for patients with OA.

F-2235

THE GERMLINE FACTOR, PRDM14, AND TSIX RNA LINK X-CHROMOSOME REACTIVATION WITH THE PLURIPOTENCY NETWORK

Bernhard Payer[1], Michael Rosenberg[1], Masashi Yamaji[2], Katsuhiko Hayashi[2], Mitinori Saitou[2], Jeannie T. Lee[1]

[1]Department of Molecular Biology, Massachusetts General Hospital/Harvard Medical School, Boston, MA, United States, [2]Department of Anatomy and Cell Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

X-inactivation is the epigenetic process by which female mammals achieve gene dosage parity with males. The inactive X is reactivated in two instances of mouse development, first in the epiblast and then in the germline. Little is presently known about mechanisms responsible for X-reactivation.

Here, we identify the noncoding Tsix RNA and the germline factor, PRDM14, as regulatory molecules. *In vivo*, X-reactivation is perturbed by loss of function of either *Tsix* or *Prdm14* in the blastocyst. *In vitro*, X-reactivation is affected by *Prdm14* deficiency in an induced pluripotent stem cells (iPSC) model. Interestingly, PRDM14 is also involved in derivation of iPSCs and postimplantation development, independently of Tsix's and PRDM14's role in X-reactivation, Finally, we identify the E3 ubiquitin ligase gene, *Rnf12*, as a direct target of PRDM14. We conclude that PRDM14 is a core factor for X-reactivation both *in vivo* and *in vitro*, and that Tsix exerts temporal control in mouse blastocysts. Collectively, these data provide a mechanistic link between X-reactivation and the pluripotency network.

F-2236

ZFP281 RECRUITS THE NURD REPRESSOR COMPLEX TO MEDIATE NANOG AUTOREPRESSION AND INHIBITS SOMATIC CELL REPROGRAMMING

Miguel Fidalgo[1], Francesco Faiola[1], Carlos-Filipe Pereira[1], Junjun Ding[1], Arven Saunders[1], Julian Gingold[1], Christoph Schaniel[1], Ihor R. Lemischka[1], Jose C.R. Silva[2], Jianlong Wang[1]

[1]Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY, United States, [2]Department of Biochemestry, University of Cambridge, Cambridge, United Kingdom

The homeodomain transcription factor Nanog plays an important role in embryonic stem cell (ESC) self renewal and is essential for acquiring ground state pluripotency during reprogramming. Enforced expression of Nanog liberates ESCs from requisite LIF dependence and accelerates somatic cell reprogramming. Understanding how Nanog is transcriptionally regulated is important to further dissect mechanisms of ESC pluripotency and somatic cell reprogramming. We previously showed that a close partner of Nanog, Zfp281, functions as a transcriptional repressor to restrict Nanog expression in mouse ESCs. Here we report that Nanog is subjected to a negative auto-regulatory mechanism, i.e. autorepression, in ESCs, and that such autorepression requires the coordinated action of Zfp281. Mechanistically, Zfp281 maintains the integrity of the NuRD repressor complex and recruits it onto the Nanog enhancer/promoter region to mediate Nanog autorepression. Functionally, Zfp281-mediated Nanog autorepression presents a roadblock for direct molecular reprogramming of somatic cells or fusion-based reprogramming of somatic and ES cell heterokaryons. Our results identify both a novel transcriptional regulatory mode of Nanog gene

expression and a transcription repressor as a novel barrier to efficient reprogramming, and shed new light into the mechanistic understanding of Nanog function in pluripotency and reprogramming.

F-2237

SIMPLIFICATION OF INDUCTION TO PLURIPOTENCY WITHOUT THE NEED OF C-MYC USING A COMPLEMENT TWO LENTIVIRAL VECTOR SYSTEM

Rick I. Cohen, Jason Huang, Anthony Parel
Biomedical Engineering, Rutgers University, Piscataway, NJ, United States

Reprogramming of somatic cells into Induced Pluripotent Stem Cells (IPSCs) can be accomplished by a variety of different methods including viral vectors hosting a popular set of four transcription factors known as Oct4, Sox2, KLF4, and C-Myc. Over the last few years the genes, types of gene transfer agents and culture techniques have been refined to approach near clinical procedures. Still the efficiency and kinetics of many protocols are low and time consuming often leading to long periods of wait-and-see. In order to streamline this process we addressed four key elements of the reprogramming paradigm; quality of cell culture starting material, optimal gene-transfer agent, use small molecule enhancing cocktail, and robustness of stem cell culture media. Firstly, we used low passage normal human foreskin fibroblasts (LifeLine Cell Technologies) derived and cultured in low serum media (FibroLife) containing enhancers of reprogramming such as ascorbic acid and hydrocortisone. Secondly, we used a Two Lentiviral Complement System without the C-Myc composed of a poly-cistronic lentiviral vector (OSK-2ARB) containing Oct4, Sox2, KLF4, and a fusion of RFP and Blasticidin-S-Deaminase, together with a second virus containing L-Myc. Thirdly, we added Valproic Acid during the first 5 days post infection, prior to gentle passaging using non-enzymatic methods onto Matrigel coated dish. Finally, we combined Knock-out serum replacer based medium with three small molecules (Sodium Butyrate, PS48, and A-83-01) for the last stage of reprogramming. Together all these optimization steps allowed us to see viral transduction efficiency, and formation of primordial IPSC colonies as early as 10 days post-infection. Clear colony formation was observed within 2 weeks, and first passage of optimized IPSCs was within 3 weeks. Our long-term goals are to remove the few remaining animal derived products and achieve robust, and rapid reprogramming and then apply this paradigm to reprogramming paradigms that leave the genome intact such as Episomal or protein mediated reprogramming.

Regeneration Mechanisms

F-2241

IMPACT OF THE INDIVIDUAL IMMUNE REACTIVITY ON HUMAN MSCS AND ENDOGENOUS BONE REGENERATION

Sven Geissler[1], Simon Reinke[1], Katharina Schmidt-Bleek[1], Kerstin Juelke[2], Michael Dahne[3], Hartwig Tony[3], Christian Meisel[4], Nadine Unterwalder[4], Petra Reinke[5], Hans-Dieter Volk[2], Georg N. Duda[1]
[A]Julius Wolff Institute & Berlin-Brandenburg Center for Regenerative Therapies, [B]Medical Immunology & Berlin-Brandenburg Center for Regenerative Therapies, [C]Julius Wolff Institute & Center for Musculoskeletal Surgery, [D]Medical Immunology & Labor Berlin, [E]Nephrology and Internal Intensive Care & Berlin-Brandenburg Center for Regenera, [1]Charite Universitaetsmedizin Berlin, Berlin, Germany

Introduction: Mesenchymal stromal cells (MSCs) are crucial for the endogenous regeneration of bone. Furthermore, bone healing is modulated by the adaptive immunity, in particular by T cells, even in absence of any infection. However, certain constraints (e.g. age, steroid therapy or diabetes) or other unknown causes delay/prevent endogenous fracture healing and lead to poor long-term outcomes. This study aimed to investigate the relation between individual's immune reactivity and MSC function with the fracture healing outcome.
Results & Discussion: Normal and delayed fracture healing patients were identified by x-ray classification after 18 weeks. Multicolor flow cytometric analyses of circulating immune T-cell subset in peripheral blood revealed a key difference between both groups. In particular, delayed fracture healing was associated with significantly higher fre-

quencies and numbers of circulating CD8+ TEMRA cells. This difference was long-lasting and reflects rather the individual's immune profile in response to life-long antigen exposure than a post-fracture reaction. Noteably, CD8+ TEMRA can be reactivated independent from antigen-presenting cells and are triggered even without T-cell receptor/antigen engagement by several cytokines delivered from activated cells of the innate immunity. Moreover, we could show that CD8+TEMRA migrate and accumulate in the human fracture hematoma and are locally the major producers of IFN-γ /TNF-α. To investigate the causal relation between the CD8+ TEMRA cells, their cytokine secretion and their effect on fracture healing, we used co-culture assays. We found that the high cytokine production of the CD8+ TEMRA cells can not be modulated by the immunoregulatory activity of MSCs. In contrast, conditioned media (CM) of sorted and ex vivo stimulated CD8+ TEMRA cells inhibited the osteogenic differentiation of human MSCs. The addition of neutralizing antibodies either against IFN-γ or TNF α only slightly increased matrix mineralization, while the combination of both almost completely restored MSC differentiation ability. Interestingly, we also found that TNF-α, but not IFN-γ, significantly reduced viability and survival of MSCs. These results indicate that the inhibitory effect of TNF α results from the induction of apoptosis while IFN-γ might directly interfere with osteogenic differentiation. The potential causal relationship between the enrichment of memory CD8+ T-cells and the pathogenesis of poor bone fracture healing, was further investigated in a mouse osteotomy model. In accordance with our in vitro results, specific CD8+ T-cell depletion by antibody therapy resulted in enhanced endogenous fracture repair (μCT evaluation), whereas a transfer of CD8+ T-cells impaired the healing process when compared to untreated animals with normal immune cell spectrum. These results provide evidence for a causal relationship between the enrichment of memory CD8+ T-cells and lower bone quality.
Conclusion: Our data demonstrate the high impact of chronically activated adaptive immunity on adult stem/progenitor cell function and endogenous regeneration processes even in the absence of any exogenous stimulus like infections. These results might open new opportunities for early and targeted intervention strategies to restore or enhance endogenous regeneration capacity. The quantification of CD8+TEMRA might present a reliable marker for the prognosis of the healing outcome.

F-2242

ACID SPHINGOMYELINASE REGULATES THE AUTOPHAGIC PROCESS IN ALZHEIMER'S DISEASE AND PARTIAL INHIBITION OF THE ENZYME IMPROVES THE PHENOTYPE OF ALZHEIMER'S DISEASE MICE

Hee Kyung Jin, **Jae-sung Bae**
Kyungpook National Univ, Daegu, Korea, Republic of

In Alzheimer's disease (AD) abnormal sphingolipid metabolism has been reported, although the pathogenic consequences of these changes have not been fully characterized. Here we show that acid sphingomyelinase (ASM) is increased in skin fibroblasts, brain homogenates and/or plasma from patients with AD and AD mice, leading to defective autophagic degradation of proteins. Partial genetic inhibition of ASM (*ASM*$^{+/-}$) in a mouse model of familial AD (*APP/PS1*) ameliorated the autophagocytic defect and improved AD pathology, including reduction of amyloid-β deposition. Genetic inhibition of ASM also led to improvement of the spatial memory impairment in AD mice. Similar effects were noted after pharmacologic inhibition of ASM using amitriptyline-hydrochloride (AMI), heterochronic parabiosis, which joined the circulations of APP/PS1 and *ASM*$^{+/-}$ mouse, and exposure of APP/PS1 mouse to plasma from *ASM*$^{-/-}$ mice. Overall, these results reveal a novel mechanism of ASM pathogenesis in AD that leads to defective autophagy, and suggests that partial ASM inhibition is a potential new therapeutic intervention for the disease.

F-2243

ARGONAUTES AND MICRORNAS IN PLANARIAN REGENERATION AND HOMEOSTASIS

Chao-Yi Chuang, Jin-Wen Huang, Chih-Kai Chang, Fang-Yi Chen, **CHIA-YING CHU**
National Taiwan University, Taipei, Taiwan

Planarians, the fresh water flat worms, are capable of regenerating lost organs or tissues. Regeneration in planarian is mediated by the adult somatic stem cells, the neoblasts, which can proliferate and differentiate for replacing whole body. MicroRNAs are the 20-24 nt small non-coding RNAs that associate with Argonaute proteins and induce RNA-mediated gene silencing in metazoan. To elucidate the function of miRNAs in Planarian regeneration we examine the expression of Argonaute proteins and other RISC components, e.g. DjCBC-1, the homologue of human RCK/p54 (DDX6), in the regenerative tissue of Dugesia Japonica. We observed that both DjCBC-1 and Djago2 are highly expressed in the regions of brain and middle dorsal line where the neoblasts locate. The expression of DjCBC-1 and DjAgo2 are elevated at post-blastema during regeneration. We also examined the miRNAs expression in regenerating region and suggested certain miRNAs may be required for the function of neoblasts. RNA interference (RNAi) of DjAgo2 abolished the regeneration of planarian and induced the degeneration process. Our current research is aimed to understand the roles of miRNAs as well as the mechanisms of DjAgo2 and chromatoid body in regeneration and homeostasis

F-2244

REGENERATION OF SYNAPSES BY CELL TRANSPLANTATION IN THE AUDITORY SYSTEM

Mingjie Tong, Albert Edge
Otolaryngology, Mass Eye Ear Infirmary, Harvard Medical School, Boston, MA, United States

Loss of both auditory hair cells (HCs) and spiral ganglion neurons (SGNs) that provide their afferent innervation cause sensorineural hearing loss. Transplantation of neural progenitors is under investigation as a therapy for restoration of hearing by replacing neurons and their synaptic connections to HCs. In an organotypic explant model using the organ of Corti, the site of the auditory hair cells and their primary connection to SGNs, we co-cultured de-afferented mouse HCs with various types of exogenous neurons to explore the mechanisms regulating auditory synaptic regeneration. Mouse SGNs sent out fibers that contacted HCs in the co-cultures, and new synapses were formed at the contacts between HCs and SGNs, as demonstrated by immunostaining of CtBP2-positive presynaptic ribbons and PSD-95-positive postsynaptic densities. The PSD-95 puncta directly faced the presynaptic CtBP2-positive foci, indicating that afferent cochlear synapses had regenerated *in vitro*. In a gain-of-function assay, we found that synaptogenesis was promoted by neurotrophins, BDNF and NT-3. In a loss-of-function assay, synaptic regeneration was impaired when glutamate release was genetically abolished in the HCs. A role for repulsive guidance molecule a (RGMa) in modulating regeneration was revealed by inhibition with a blocking antibody against the guidance protein. Furthermore, mouse embryonic stem (ES) cell-derived neuronal progenitors innervated HCs with cochlea-like afferent synapses when co-cultured with de-afferented HCs, comprising a clinically feasible future approach to SGN replacement by stem cells.
Supported by NIDCD grants R01 DC007174 and P30 DC05209.

F-2245

TRANSPLANTATION OF BONE MARROW MONONUCLEAR CELLS PROMOTES NEUROPROTECTION AND MODULATION OF INFLAMMATION AFTER STATUS EPILEPTICUS IN MICE

BRUNO SOUZA[1], MARCOS MAURICIO TOSTA LEAL[1], Zaquer Costa-Ferro[2], Carine Azevedo[1], Thiago Carvalho[3], Carla Martins Kaneto[3], RICARDO RIBEIRO DOS SANTOS[3], Milena Soares[1]
[1]Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil, [2]PUC-RS, Hospital, Salvador, Brazil, [3]Centro de Biotecnologia e Terapia Celular, Hospital São Rafael, Salvador, Brazil

Background: Status epilepticus (SE) is a severe clinical manifestation of epilepsy. Neuronal loss and inflammatory processes contribute to the pathogenesis of temporal lobe epilepsy. Bone marrow mononuclear cells (BMMC) are candidate for the development of cell therapies for neurological disorders for their neuroprotective and antiinflammatory effects. Here we investigated the effects and mechanisms of action of transplanted BMMC after SE induced in mice by administration of pilocarpine. Methods: Mice injected with pilocarpine for SE induction were random-

ized and distributed into three groups: saline-treated controls, transplanted intravenously with 1x106 BMMC isolated from GFP transgenic mice, and injected with BMMC lysate. Mice were euthanized at different time points after SE for cell tracking, neuronal counting in hippocampal subfields and analysis of cytokine production in the serum and brain. Results: BMMC were found in the brain shortly after transplantation and their numbers decreased with time. A reduced neuronal loss in the hippocampus after SE was found in mice treated with BMMC and BMMC lysate when compared to saline-treated, SE-induced mice. Moreover, the gene expression of the inflammatory cytokines IL-1β, TNF-α, IL-6, as well as NOS2, were decreased in the brain after injection of BMMC and to a less extent, of BMMC lysate, when compared to SE-induced controls. Increased levels of anti-inflammatory cytokines TGF-β, IL-10 and IL-4 were found in the sera of mice treated with BMMC when compared to SE-controls. The expression of genes associated with alternative activation of microglia and macrophages MRC1, Chi3l3, ARG1, CCl22, CCl17 was increased in all groups after SE. Conclusion: BMMC transplantation promotes neuroprotection and have anti-inflammatory effects after SE in mice, possibly due to a paracrine action.

F-2246

NANOFIBROUS CARDIAC PATCH OF PCL/GELATIN CARRYING MSCS IS EFFECTIVE FOR PREVENTING EXPANSION OF THE LEFT VENTRICLE AND REPAIRING INFARCTED MYOCARDIUN AFTER MYOCARDIAL INFARCTION

Yu-Zhen Tan[1], Qiang-Li Wang[1], Hai-Jie Wang[1], Dan Kai[2], Seeram Ramakrishna[2]

[1]Shanghai Medical School of Fudan University, Shanghai, China, [2]Nanoscience and Nanotechnology Initiative, Faculty of Engineering, National University of Singapore, Singapore, Singapore

Myocardial infarction becomes the leading cause of congestive heart failure and death all over the world. Transplantation of stem cells is promising therapy for myocardial infarction. However, outcomes of systemically and locally transplanted stem cells are undesirable because lose and poor survival of the cells. Recently, we fabricated a nanofibrous cardiac patch with PCL/gelatin (PG) loaded mesenchymal stem cells (MSCs), and then examined its effectiveness in repair of the infracted myocardium. Mixture of poly(ε-caprolactone) and gelatin were electrospun into nanofibrous membrane, then MSCs isolated from rat marrow were seeded on it. Compatibility of the cells and nanofibers was evaluated with a scanning electron microscope and transmission electron microscope. Survival of the cells on PG nanofiber membranes was decided in a hypoxia model. The rat models of myocardial infarction were established by ligation of left anterior descending coronary artery. At 1 week after myocardial infarction, the PG/MSC cardiac patches were implanted onto the epicardium at infarcted region of rat MI models. The fates of the transplanted cells were traced with GFP gene transfection and Y chromosome probe. The cells spread and grew well on the membrane. After incubation under hypoxia condition, survival number of the cells on the nanofibrous membranes was higher than that in control group, and apoptosis and necrosis cells decreased. In implantation onto the epicardium, PG/MSC cardiac patch prevented expansion of the infarcted wall of the left ventricle, and improved significantly cardiac functions. After implantation for four weeks, PG was degraded partially. There are many cells expressing cTnT and Cx43 beneath the epicardium, and density of microvessels in peri-infarcted and infarcted region was increased significantly in PG/MSC cardiac patch group compared with control and PG cardiac patch groups. GFP^+ and Y chromosome-positive cells located in the patch or migrated into the subepicardium and superficial layer of the myocardium, some of them differentiated into cardiomyocytes or endothelial cells. Scar area reduced significantly in PG/MSC cardiac patch group compared with control and PG cardiac patch groups. Y chromosome-positive cells expressing CD31 were observed on the wall of microvessels, indicating the cells participated directly in angiogenesis of infarcted tissue. Lymphangiogenesis was not obvious. These results demonstrate that PG/MSC cardiac patch can prevente expansion of left ventricle wall, protect effectively cardiac activities and promote cardiomyogenesis and angiogenesis. Implantation of PG/MSC cardiac patch is novel therapy for repair of the myocardium and improvement of cardiac functions after MI.

F-2247

HUMAN iPS CELL DERIVED CARDIAC PROGENITOR CELLS FOR CARDIAC REGENERATION

Masafumi Takeda, Jun K. Yamashita
Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

Background: Cardiac regeneration is highly attractive because heart failure is the leading cause of death in developed countries due to limited ability for self-repair of the heart. Recently, several kinds of cell sources such as bone marrow-derived stem cells, skeletal myoblasts, and endogenous cardiac stem cells have been investigated for cardiac regeneration. However, these cell sources have not achieved satisfactory results. Considering the strategy of cell transplantation, hiPS cells-derived cardiac progenitor cells would be a promising cell source. Recently, our group has developed a stepwise differentiation protocol for hiPS cells to cardiomyocytes based on a high density monolayer culture for human ES cells with some modifications (Modified DD protocol). This protocol is amenable to identify and obtain cardiac progenitor cells with high and selective cardiogenic potential. In this study, we attempted to monitor the changes of several kinds of surface markers to assess the cardiomyocyte differentiation process and identify cardiac progenitor population derived from hiPSCs.
Methods and Results: We assessed mesoderm induction with KDR and PDGFRα expression under modified DD protocol. We could observe KDR+ PDGFRα+ (DP) population clearly emerged from day 4 and gradually shifted into KDR- PDGFRα+ (SP) population. We could detect cardiac troponinT-positive cells at day 7. To identify cardiac progenitor cells, we examined the potential of DP or SP population emerged from day4 to day6 for producing three cardiovascular lineages such as cardiomyocytes, pericytes, and endothelial cells. We sorted DP or SP population and re-cultured them in a serum-free culture (RPMI containing B27 supplement alone) or either with 50ng/ml VEGF or 10ng/ml PDGFBB for 2 weeks. DP population from day4 and 5 yielded cardiomyocytes with about 75% and 85% efficiency, respectively. DP and SP populations at day6 demonstrated a high cardiogenic potential (cardiomyocytes with over 90% efficiency, pericytes with 3-9%, and endothelial cells with less than 1%). The effect of VEGF on producing endothelial cells from DP population at day6 was still remained (endothelial cells with about 4% efficiency), although its effect from SP population was almost lost. PDGFBB had no effect on inducing pericytes from both DP and SP populations.
Conclusions: We demonstrated KDR- PDGFRα+ (SP) population was strongly committed to cardiomyocytes and considered to be as a cardiac progenitor-enriched population. We will further examine this cardiogenic potential in vivo and apply the strategies with this population for cardiac regeneration. We are also trying to identify a core progenitor subset within the SP population.

F-2248

ELUCIDATING THE ROLE OF SPARCL1 IN CARDIO PROTECTION IN AN IN VITRO MYOCARDIAL INJURY MODEL

Siti Ismail, Claire Kavanagh, O'Brien Timothy, Barry Frank
Regenerative Medicine (REMEDI), National University of Ireland in Galway (NUIG), Galway, Ireland

Background
There is great interest in mesenchymal stem cells as pharmacological mediators in tissue repair. Paracrine effects, exerted through the release of biologically active factors, are the key mechanisms mediating MSC-based therapy in many diseases. The main target of stem cell-based therapy in cardiac disease is the ability of the myocardium to regenerate, revascularize and improve of cardiac function. Several observations indicate that MSCs are activated by the ischemic environment of the infarct resulting in secretion of a cascade of factors with anti apoptotic, immunomodulatory, cytoprotective and pro-angiogenic potential. Using an engrafted myocardial infarction model several factors abundantly secreted by MSCs were identified by gene expression analysis and mass spectrometry. In this study, we focus on one novel and promising therapeutic factor known as SPARCL1. SPARCL1, is an extracellular matrix glycoprotein and has been described in many cellular processes. This is the first study describing the cardio-protective effect of SPARCL1. The aim of the study was to elucidate the role and potential mechanism of SPARCL1 in cardio protection and MSC survival in an in vitro myocardial infarction or ischemia.
Methods
The cardiomyocytes/MSC injury model was established by exposing rat primary neonatal cardiomyocytes and bone marrow derived MSC to a hypoxic environment (0.5% oxygen) in the absence of serum. Furthermore, to mimic

acute myocardial infarction in vivo, a neonatal cardiomyocyte cell line was subjected to oxidative injury by hydrogen peroxide. Viability, cytotoxicity and caspase activity were monitored to measure the cyto protective effects of SPARCL1. Genes with potential cardio-protective effects were analysed by quantifying at mRNA and protein levels.
Results
Using the in vitro model of oxidative stress, SPARCL1 treatment resulted in significant increase of cell viability and a decrease in apoptosis for up to 6 hours post treatment. These observations were confirmed by gene and protein expression which demonstrated elevated expression of anti apoptotic genes Bcl-2 and Birc3, and reduction of anti fibrotic genes Col1, Col3 and TGF-β. SPARCL1 protein also exhibits pro-angiogenic properties by increasing tubular formation in an in vitro matrigel angiogenesis assay and increased capillary formation in vivo. Interestingly, the capillary formation was significantly higher in lower dose (100ng/ml) compared to the higher dose (400ng/ml) indicating a threshold effect. SPARCL1 treatment during induced oxidative stress demonstrated a significantly increased expression of pAKT compared to the non-treated group, indicating the involvement of PI3K/AKT pathways in the survival of cells in oxidative stress injury.
Discussion and conclusion
In this study, we demonstrate that SPARCL1 is a major component of the in vivo secretome of MSCs in ischemic myocardium. SPARCL1 protected cardiomyocytes via inhibition of apoptosis and reduced fibrosis in an in vitro oxidative-induced myocardial injury model. These findings supported the hypothesis that SPARCL1 is a promising candidate for therapies aimed at reducing myocardial injury and resulting cardiac dysfunction. The present study defined the mechanistic properties of SPARCL1 in the context of cardio protection. This work highlights the new perceptive that therapeutic effects are mediated via the secretome and will overcome current limitation of cells delivery in cardiac regenerative therapy.

F-2251

STRATEGIES TO ENHANCE THE EFFICIENCY OF ENDOTHELIAL PROGENITOR CELLS-BASED THERAPY IN PHYSIOLOGICAL AND PATHOLOGICAL WOUND HEALING

philippe FOUBERT[1], claire SQUIBAN[2], valerie BUARD[2], valerie holler[2], marc BENDERITTER[2], Jean Sebastien SILVESTRE[3], bernard LEVY[1], gerard TOBELEM[1], **radia TAMARAT**[2]
[1]IVS, PARIS, France, [2]IRSN, FONTENAY AUX ROSES, France, [3]INSERM, HOPITAL HEGP, France

Endothelial progenitor cell (EPC) transplantation has beneficial effects for therapeutic neovascularisation. We therefore assessed the effect of a therapeutic strategy based on EPC administration in the healing of radiation-induced damage. To improve cell therapy for clinical use, we used pre-treatment with ephrin B2-Fc (Eph-B2-Fc) or and co-administration with smooth muscle progenitor cells.
At Day 3, EPCs promoted dermal wound healing in both non-irradiated and irradiated mice by 1.2 - and 1.15 -fold, respectively compared with animals injected with PBS. In addition, EPCs also improved skin blood perfusion and capillary density in both irradiated and non-irradiated mice compared with PBS-injected animals. We also demonstrated that activation with ephrin-B2-Fc increased wound closure by 1.6-fold compared with unstimulated EPC in non-irradiated mice. Interestingly, the beneficial effect of Eph-B2-Fc was abolished in irradiated animals. In addition, we found that Eph-B2-Fc stimulation did not improve EPC- induced vascular permeability or adhesiveness compared to unstimulated EPC cells. We hypothesised that this effect was due to high oxidative stress during irradiation leading to inhibition of EPC beneficial effect on the vascular function. In this line, we demonstrated that in irradiated conditions, N-acetyl-L-cysteine treatment restored the beneficial effect of EPC stimulation with Eph-B2-Fc in wound healing process.
In conclusion, stimulation by Eph-B2-Fc improved the beneficial effect of EPCs in physiological conditions and irradiated conditions only in association with antioxidant treatment. Additionally, co-therapy was beneficial in pathological conditions.

F-2252

MEGAKARYOCYTES SERVE AS A STRESS RESPONSE NICHE TO SUPPORT HEMATOPOIETIC STEM CELL RECOVERY FOLLOWING MYELOABLATIVE THERAPY

meng zhao[1], John M. perry[1], xi C. He[1], Chu-Xia Deng[2], linheng Li[1]
[1]stowers institute for medical research, Kansas city, MO, United States, [2]The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), Bethesda, MD, United States

Understanding how hematopoietic stem cells (HSCs) recover from myeloablative therapy is essential for leukemia treatment and successful bone marrow (BM) transplantation. HSC niches are critically important for HSC regulation and maintenance; however, little is known about the identity and function of niche cells supporting initial HSC recovery in response to myeloablative therapy. Recently, we reported that fibroblast growth factor (FGF) signaling expedited post-injury recovery of the mouse hematopoietic system by promoting proliferation and facilitating mobilization of HSCs. Interestingly, megakaryocytes (MKs) co-expanded with HSCs in vitro, underwent FGF-FGF receptor (FGFR) signaling-dependent expansion near the surrounding blood vessel, and produced FGFs *in vivo* post 5-fluouracil (5FU) treatment, which indicated the role of MKs in supporting HSC recovery. Here, we investigated the role of MKs in supporting HSC recovery post myeloablative therapy. Using a Cre-inducible diphtheria toxin receptor genetic model, we observed that Mk depletion led to a reduced HSC *ex vivo* expansion (54% decrease) and loss of *in vivo* engraftment ability (91% decrease) post culture. Ablation of Mks *in vivo* elevated HSC number (2.6-fold increase) under homeostasis, but caused a 45% decrease of functional HSCs in BM and subsequent HSC mobilization (3-fold increase) to spleen post 5FU. Since the spleen may support expansion of mobilized HSCs, we ablated MKs in splenectomized mice and observed an 80% decrease of HSC recovery in BM post 5FU compared to splenectomized mice without MK depletion. These results suggest that Mks function as critical niche to support HSC recovery from myeloablative therapy in BM, while spleen functions as a less optimal backup niche to mediate HSC recovery. Mechanistically, we found FGFR1 was up-regulated in Mks post BM damage, and conditional knockout of *Fgfr1* in Mks suppressed HSC post-injury recovery in BM (72% decrease), but did not affect the number of functional HSCs in spleen. This study demonstrates that MKs serve as a stress response niche to support HSC recovery following myeloablative therapy.

F-2253

THE EPIGENETIC REGULATION OF RENAL STEM CELL NUMBER IN ZEBRAFISH

Dongdong Ma, Robert I. Handin
Hematology Division, Brigham and Women's Hospital, Boston, MA, United States

We have previously reported that normal renal growth and regeneration after injury is regulated by an adult stem cell resident in the zebrafish mesonephros (Nature 470:95-100). In these experiments lhx-GFP+ stem cells were obtained from Tg(lhx1:GFP;cdh17:mCherry) zebrafish, transplanted into irradiated wild type recipients and nephron generation assessed by examining the number of mCherry-positive nephrons. Subsequently, we have observed that the number of lhx1-GFP+ cells in the kidneys of these transgenic donors increases 8 fold when the fish have been exposed to several classes of histone deacetylase inhibitors (HDACi). We have seen a response with 4-mPTB, SAHA, Merck60 and Mer161 inhibitors. HDACis synergize with nephrotoxins like gentamycin to cause a further increase in stem cell number. Since the mesonephros serves as the hematopoietic organ in zebrafish, we also examined the effect of HDAC inhibitors on CD41-GFP^{lo} cells which we have shown are hematopoietic stem cells and found that drug treatment did not increase the number of CD41-GFP^{lo} cells in kidney marrow. We next examined the effect of HDAC inhibitors on fish of varying ages. HDAC inhibitors increased lhx1:GFP cell number 8 fold in young (three month) fish but only two fold in aged (two year) fish. We believe these observations are of general interest, as there are few other examples of epigenetic regulation of stem cell proliferation. In addition, the profound effect of aging on stem cell proliferation in response to HDAC inhibitors has not, to our knowledge, been previously reported. The zebrafish model we have developed will give us an opportunity to dissect the mechanism and pathway(s) that regulate stem cell proliferation and determine whether the effects are cell intrinsic or involve changes in systemic (humoral) factors or changes in the renal stem cell niche.

F-2254

SOX2 DEFINES AND REGULATES ADULT NEURAL CREST PRECURSORS TO PROMOTE TISSUE REPAIR

Adam P.W Johnston, Sibel Naska, Karen Jones, Hiroyuki Jinno, Freda D. Miller
Developmental and Stem Cell Biology, The Hospital for Sick Children, Toronto, ON, Canada

Potential insights into the mechanisms regulating mammalian tissue repair come from studies of animals like amphibians and reptiles which can regenerate limbs, tails, and even the spinal cord. One major conclusion of these studies is that tissue regeneration requires intact nerve innervation, leading to the idea that peripheral nerves might regulate tissue repair in mammals, a possibility with important implications, since every tissue in the body receives nerve innervation. Here, we have tested this idea in mice, focusing upon Sox2-positive neural crest precursors and skin repair. Using mice that express EGFP from the Sox2 locus, we show that in adult skin, Sox2 is expressed in nerve terminal-associated neural crest/Schwann cell precursors (NCPCs) around the hair follicle bulge and, that following skin injury, it is induced in NCPCs within cutaneous nerves. At later times post-injury, Sox2-positive cells are scattered throughout the healing dermis. Lineage tracing with Wnt1-Cre;TdTomatofl/fl and Dermo1-Cre;TdTomatofl/fl mice, which label neural crest and mesenchymal progeny respectively, show that these cells are all neural crest-derived cells with the phenotype of NCPCs. Moreover, lineage tracing with a mouse where CreERT2 is knocked-in to the Sox2 locus showed that most of these Sox2-positive NCPCs migrate into the regenerating dermis from adjacent cutaneous nerves. These Sox2-positive NCPCs are functionally important, since skin repair is deficient in mice where Sox2 is haploinsufficient, and acute deletion of Sox2 prior to injury causes a decrease in NCPCs in the wound and a coincident decrease in skin repair. Thus, Sox2 regulates the function of NCPCs following tissue injury, and decreases in the Sox2-dependent NCPC injury response causes aberrant skin repair. Since almost all mammalian tissues receive peripheral nerve innervation, these data suggest that nerve-derived NCPCs may play a broad role in mammalian tissue repair and regeneration.

F-2255

AURORA-A KINASE REGULATES MAMMARY EPITHELIAL CELL FATE BY DETERMINING MITOTIC SPINDLE ORIENTATION IN A NOTCH-DEPENDENT MANNER

Joseph Regan[1], Tony Sourisseau[2], Kelly Soady[1], Howard Kendrick[3], Afshan McCarthy[1], Chan Tang[1], Keith Brennan[4], Spiros Linardopoulos[1], Donald White[1], Matthew J. Smalley[3]
[1]The Institute of Cancer Research, London, United Kingdom, [2]Institut Gustave Roussy, Paris, France, [3]European Cancer Stem Cell Research Institute, Cardiff, United Kingdom, [4]Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom

Cell fate determination in the progeny of mammary epithelial stem/progenitor cells remains poorly understood. Here, we have examined the role of the mitotic kinase AuroraA (AurkA) in regulating the balance between basal and luminal mammary lineages. We find that AurkA is highly expressed in basal stem cells and to a lesser extent in luminal progenitors. wtAurkA expression promoted luminal cell fate but expression of an S155R mutant blocked proliferation, promoted basal fate and inhibited serial transplantation. The mechanism involved regulation of mitotic spindle orientation by AurkA and the positioning of daughter cells after division. Remarkably, this was Notch-dependent, as a Notch inhibitor blocked the effect of wtAurkA expression on spindle orientation and instead mimicked the effect of the S155R mutant. These findings directly link AurkA, Notch signalling and mitotic spindle orientation and suggest a novel mechanism for regulating the balance between luminal and basal lineages in the mammary gland.

F-2256

HMGA2 EXPRESSION IS REQUIRED FOR THE HIGH SELF-RENEWAL ACTIVITY OF FETAL HEMATOPOIETIC STEM CELLS

Michael R. Copley, David G. Kent, Claudia Benz, Sonja Babovic, Stefan Wohrer, David Q. Treloar, Chris W. Day, Keegan M. Rowe, Connie J. Eaves
Terry Fox Lab, BC Cancer Agency, Vancouver, BC, Canada

Hematopoietic stem cells (HSCs) are rare cells defined by their ability to produce clonal populations of mature blood cells for the lifetime of the organism. This capacity reflects their possession of mechanisms that enable them to execute unlimited divisions without activating the hematopoietic differentiation programs they are poised to initiate _ a property known as self-renewal. The first HSCs to emerge in the mouse embryo display very high self-renewal activity, which is later down-regulated shortly after birth. In a recent study, we found that the high-mobility group AT-hook 2 (*Hmga2*) gene is expressed at an ~30-fold higher level in 40-50% pure suspensions of fetal as compared to adult HSCs. Due to its differential expression pattern and the known role of *Hmga2* as both a transcription factor and a chromatin regulator, we hypothesized that increased *Hmga2* expression may be a determinant of the elevated self-renewal activity characteristic of fetal HSCs. To test this hypothesis, we first compared the composition of the hematopoietic compartments of *Hmga2*$^{-/-}$ (knock-out; KO) and *Hmga2*$^{+/+}$ (wild-type; WT) mice. Although we found no difference in the absolute numbers of HSCs and progenitors in the fetal liver of KO and WT mice, in the adult, HSCs and progenitors are present at 5-fold lower levels ($P < 0.05$) in KO bone marrow. To compare the self-renewal potential of fetal KO and WT HSCs, we examined their expansion kinetics in sublethally irradiated congenic *Hmga2*-WT W^{41}/W^{41} recipients. Six weeks following the transplantation of 1.7×10^5 KO or WT FL cells (~4-5 HSCs), we measured a significant reduction ($P < 0.05$) in the frequency of donor-derived Lin-Sca1+c-Kit+ cells in the marrow of recipients of KO versus WT cells. To test if this difference would reflect a similar effect on HSCs, we transplanted secondary mice with matched, decreasing aliquots of cells harvested from the respective primary 6-week recipients. The secondary recipients of the highest dose of cells from the primary recipients showed a reduced KO cell-derived chimerism in both the myeloid and lymphoid lineages, as compared to corresponding WT cell contributions. Limiting dilution analysis of the remaining secondary recipients showed that the initial fetal KO HSCs had expanded significantly less (13-fold; $P = 10^{-11}$) than the fetal WT HSCs during the first 6 weeks in the primary recipients. Interestingly, the extent of HSC expansion obtained in these experiments, and the difference between KO and WT HSCs, are remarkably similar to the results obtained when WT adult and fetal HSCs are compared using the same experimental design. Our data thus support a model in which the elevated expression of *Hmga2* in fetal HSCs enables them to divide with a higher probability of self-renewal than is later operative in adult HSCs.

F-2258

SKP$_S$-LIKE PROGENITOR CELLS ISOLATED FROM MOUSE DIGIT ARE ASSOCIATED WITH DIGIT TIP REGENERATION

Jianghai Chen, Zhenbing Chen, Yizhen Huang, Xiaocheng Lu
Hand Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei Province, China

Regrowth of mammal digit tip has been reported in mice and humans after amputation through the distal interphalangeal level. Although it had been assumed that a unique pluripotent cell population might give rise to all regenerated tissues, several studies have demonstrated that lineage-restricted progenitor cells mediate regeneration of mouse digit tip after amputation injury recently. The objective of this study was to isolate stem/progenitor cells resided in digit tip and identify the putative function of these cells. Here we used skin-derived precursors (SKPs) culture condition to isolate and expand potential stem/progenitor cells from mouse digit tip. Under such condition, SKPs-like spheres were observed after seven days in culture. Microarray and real-time PCR showed that the cells from these spheres presented similar molecular characteristics to SKPs. Immunocytochemistry revealed that most of sphere cells were positive for both Sox2 and Nestin staining. Additionally, the cells were capable of self-renewal and multipotency in vitro. Moreover, we showed that endogenous Sox2$^+$/Nestin$^+$ cells in digit tip were mobilized after amputation injury. Transplantation of GFP-digit tip derived sphere cells confirmed that donor cells integrated in digit tip regeneration. Collectively, these results demonstrate that digit tip derived sphere cells are SKPs-like cells and can functionally implicate in digit tip regeneration after amputation.

F-2261

NON-PLATELET RNA-CONTAINING PARTICLE-DERIVED CELLS REGENERATE KIDNEYS

Wuyi Kong
Khasar Medical Technology, Beijing, China

Kidney regeneration by non-platelet RNA-containing particle-derived cells

Wuyi Kong[1]*, Mu Nuo[1], Xiao Ping Zhu[1], Xiu Juan Han[1], Xian Wang[2]

[1]Beijing Khasar Medical Technology, Beijing 100085, China

[2]Department of Physiology, Beijing University, Beijing 100083, China

We found a group of non-platelet RNA-containing particles (NPRCPs) in mouse blood. The NPRCPs are about 1 to 5 m, have a thin bi-layer membrane, contain short RNAs and microRNAs, and express Oct4, sox2, and DDX4. To confirm the function of NPRCPs *in vivo*, we examined ischemia-damaged mouse kidneys from day 1 to week 6 after tail-vein transplantation with NPRCPs. Within 1 day, a large amount of NPRCPs migrated to the renal calyces areas, damaged glomerulus, and duct tubules via extravasation. During regeneration, NPRCPs fuse into large cellular patch-like structures that further regenerated kidney tubules. In addition NPRCPs can became tiny nucleated cellular structures that further trans-differentiated into cells in interstitial spaces or connective tissues. Non-nucleated NPRCPs can also arranged into capillary net structures before further regeneration into cells in the glomerulus. Our data demonstrate that mouse kidney tissues damaged by ischemia can be regenerated by NPRCPs by various differentiation patterns.

F-2262

COOPERATIVE ACTIVATION OF WNT/β-CATENIN AND PI3K/AKT EXPANDS PRIMITIVE HUMAN CORD BLOOD-DERIVED HEMATOPOIETIC STEM CELLS

John M. Perry[1], Xi C. He[1], Sunil Abhyankar[2], Sheng Ding[3], Linheng Li[1]

[1]Stowers Institute for Medical Research, Kansas City, MO, United States, [2]University of Kansas Medical Center, Kansas City, KS, United States, [3]Gladstone Institute of Cardiovascular Disease, San Francisco, CA, United States

Hematopoietic stem cell (HSC) self-renewal and differentiation are mutually exclusive events critical to the maintenance of long-term and short-term hematopoiesis, respectively. Recovery from myeloablative therapy requires rapid production of mature hematopoietic lineages, but this can conflict with the requirement for maintaining lifelong hematopoiesis through primitive HSC.

Using the murine system, we previously established a cellular and molecular basis for self-renewal. Specifically, activation of β-catenin blocked differentiation of proliferating HSCs but resulted in apoptosis. In contrast, activation of the PI3K pathway allowed survival of proliferating HSCs but increased differentiation. However, both pathways together cooperate to allow survival of proliferating HSCs that are blocked from differentiating--three events which together define self-renewal. This knowledge was then used to develop an *ex vivo* expansion protocol for HSCs using unsorted mononuclear cells (MNCs) as the culture input. Using HSC expansion media which pharmacologically activates both the β-catenin and PI3K pathways, MNC cultures evolved from a population of mostly mature cells to a population of approximately 15% HSCs, 15% myeloid progenitor cells and 70% megakaryocyte progenitors. In addition to a 100-fold expansion of primitive HSCs, this protocol also expanded cells involved in short-term repopulation of the hematopoietic system.

Being readily available and having lower donor-host matching requirements, umbilical cord blood (UCB) is an ideal source of HSCs for therapy but is not widely used due to limited cell numbers in single cords and severely delayed engraftment. Based on our murine studies, we tested whether cooperative activation of the β-catenin and PI3K pathways could similarly expand HSCs derived from human UCB and whether our unique culture methods may also enhance short-term engraftment kinetics.

We found that pharmacological activation of both the β-catenin and PI3K pathways together synergistically expands phenotypic HSCs isolated from UCB by 82-fold, on average. To test for primitive HSC function, we used serial trans-

plant assays of UCB cells into NOD/SKID/IL2Rγ recipients and observed a 7-fold increase in human engraftment levels for sorted, cultured HSCs vs. sorted uncultured HSCs in secondary transplant recipients (3.1 ± 4.0 vs. 21.5 ± 14.9%). Unsorted cultures of MNCs containing an equivalent number of HSCs yielded a 19-fold increase vs. uncultured, sorted HSCs (58.5 ± 9.3%). While all recipients of uncultured MNCs (n=30) succumbed to Graft-versus-Host Disease (GvHD) by 25 days post-primary transplant, all recipients of cultured MNCs survived until sacrificed at 16 weeks. In addition, recipients of cultured MNCs exhibited a 3-fold increase in platelet counts and overall myeloid engraftment at 15 days post-transplant compared to uncultured or cultured, sorted HSCs. These results show that our *ex vivo* culture system expands primitive HSCs and cells involved in short-term engraftment while also protecting the recipients from GvHD.
Using defined media and supporting cells derived only from the same cord unit with no permanent genetic manipulation, our human HSC expansion protocol is ideal for clinical translation. Furthermore, it is expected to solve the most significant obstacles to wide-spread use of UCB including lack of sufficient HSCs from single cord units and delayed kinetics of hematopoietic recovery.

Tissue Engineering

F-2265

COMPARISON OF A COLORIMETRIC AND INDIRECT METHOD TO EVALUATE THE ADHESION OF STEM CELLS ON NANOFIBER SCAFFOLDS

Fernanda Zamboni[1], Daikelly I. Braghirolli[1], **Patricia Pranke**[2]

[1]Hematology and Stem Cell Laboratory, Faculty of Pharmacy - Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [2]Hematology and Stem Cell Laboratory, Faculty of Pharmacy - Federal University of Rio Grande do Sul; Stem Cell Research Institute, Porto Alegre, Brazil

In tissue engineering, adhesion is the first step of interaction between cells and the biomaterials/scaffolds. Consequently, this parameter is widely studied as a way of assessing the bioactivity of scaffolds. In literature, different methods are used to assess this first contact of cell to scaffold. The purpose of this study, therefore, has been to compare two methods of evaluation of stem cell adhesion onto scaffolds. Colorimetric assay, based on the reduction of tetrazolium salt (MTT) by mitochondrial enzymes, was compared to the indirect method,based on the number of cells that did not attach on the scaffolds. For this comparison, poly(DL-lactic-co-glycolic acid) (PLGA) nanofiber scaffolds produced by electrospinning were used. The scaffolds were placed in a 24 well plate and sterilized by ultraviolet radiation (2h). The cells were isolated from human deciduous dental pulp and cultured in DMEN media supplemented with 10% fetal bovine serum and 1% antibiotic, in a humidified atmosphere at 5% CO2 and 37°C. On the fifth passage, the isolated cells were characterized as mesenchymal stem cells (MSCs). The cells were able to differentiate into adipocytes, osteoblasts and chondrocytes. When the phenotypic profile was analyzed, the cells were positive for CD29 (99.6%), CD44 (74.0%), CD73 (99.5%) and CD90 (99.6%) antigens, whereas a low expression was detected for CD14, CD34, CD45, CD184 and HLA-DR (all $\leq$ 0.1%). Following this, the MSCs between the 6th until the 10th passage were seeded onto the scaffolds at a density of 3 x 105 cells/sample. After 3h incubation, the cellular adhesion tests were realized (n=5, triplicate). For the indirect method, the medium containing non adherent cells was removed and centrifuged at 300g for 5 min. The pellet was re-suspended in a known volume of PBS buffer and the MSC were counted in a Neubauer chamber. The number of adhered cells on the scaffolds was calculated from the difference between the number of cells initially seeded on the biomaterial and the number of non-adherent cells, counted in a Neubauer chamber. For the colorimetric assay, the culture medium was removed and 200 µL MTT solution (0.25 µg/mL) was added. After 2 hours, the MTT was removed and replaced by 400 µL dimethyl sulfoxide (DMSO). The absorbance of the DMSO containing soluble formazan blue was read at 560 nm with 630 nm reference. The number of adhered stem cells on the scaffolds was obtained using a standard MTT absorbance curve for MSCs of known concentration. The number of adhered cells was similar for both methods: 27 x 103 ± 1.4 and 21 x 103 ± 7.9 cells/scaffold for the indirect and colorimetric method, respectively. No statistical difference (Student´s t test) was found. Although the two methods have shown a similar average number of adhered cells on the scaffolds, the colorimetric test showed a higher standard deviation than the indirect method. Additionally, the

colorimetric method has a higher cost than the indirect method and it requires a larger number of cells because it is necessary to produce a standard absorbance curve so that the number of cells can be calculated. The indirect method was shown to be a simple technique but it requires a trained operator to count the cells. The results of this work suggest that both methods can be used in the assessment of scaffold cell adhesion. The method choice should be based on the benefits of each test in addition to the conditions of each laboratory. Moreover, the use of both methods provides additional results, rendering the analysis more accurate.

F-2266

DEVELOPMENTALLY GUIDED PROTOCOL IMPROVES IPS CELL TO RPE DIFFERENTIATION EFFICIENCY AND GENERATES FUNCTIONAL RPE TISSUE

Kapil Bharti[1], Janine Davis[1], Barbara Corneo[2], Jason Silver[1], Qin Wan[1], Kiyoharu Miyagishima[1], Vaisakh Mohanrajan[1], Ruchi Sharma[1], Fang Hua[1], Arvydas Miminishkis[1], Sally Temple[2], Sheldon Miller[1]

[1]National Eye Institute, National Institutes of Health, Bethesda, MD, United States, [2]New York Neural Stem Cell Institute, Rensselaer, NY, United States

Age-related macular degeneration (AMD) is the leading cause of blindness in the US, affecting more than 10 million people. It is thought that disease symptoms are initiated by degeneration of the retinal pigment epithelium (RPE), a monolayer of cells located in the back of the eye adjacent to the photoreceptor cell layer. The induced pluripotent stem (iPS) cells technology has provided a hope for AMD treatment. iPS cells can be used to study disease mechanisms in vitro and can be used to develop a cell-based therapy. To fully harness this potential of the iPS cell technology, there is a need to develop differentiation protocols that are robust, efficient, and reproducible across different patient derived iPS cell lines. RPE generated from iPS cells need to be functionally authenticated. The aim of this study is to improve iPS cell to RPE differentiation efficiency and to functionally authenticate generated RPE cells.
To optimize iPSC to RPE differentiation protocol, we developed a human iPS cell reporter line that expresses RPE-specific GFP. The GFP expression in this iPS cell line strongly correlates with the induction of RPE phenotype when differentiated. Using mouse models we have demonstrated that the GFP expression is dependent on two RPE-specific transcription factors PAX6 and MITF. We used existing knowledge of RPE development to test combinations of growth factors FGF, BMP, WNT, SONIC, and TGF-family for their ability to improve GFP signal and RPE signature genes expression in iPS cells differentiating towards RPE. The newly optimized protocol differentiates up to 100% iPS cells into RPE-like cells, as judged by the GFP and RPE-marker expression. This method increases, by several folds, the expression of MITF and PAX6 in cells that are progenitors to the RPE lineage. Differentiated cells are grown on semi-permeable membranes to generate a monolayer tissue, which is functionally authenticated using molecular and physiological assays. RPE generated from iPS cells using this new method closely resembles primary human RPE in its molecular and physiological properties. This protocol is easily amenable to current Good Manufacturing Practices, generates authentic RPE from patient-derived iPS cells, and can generate large batches of authentic RPE for high throughput screens.

F-2267

ESTABLISHMENT OF THREE DIMENSIONAL CULTURE SYSTEM USING POLY(ETHYLENE) GLYCOL FOR MOUSE PREANTRAL FOLLICLE IN VITRO DEVELOPMENT

Jong Il Ahn[1], Gil Ah Kim[2], Ji Yeon Ahn[3], Jeffrey Alan Hubbell[4], Seung Tae Lee[5], Jeong Mook Lim[1]

[1]Major in Biomodulation WCU program, Seoul National University, Seoul, Korea, Republic of, [2]Dept of Agriculture Biotechnology, Seoul National University, Seoul, Korea, Republic of, [3]Cancer Research Institute, Seoul National University, Seoul, Korea, Republic of, [4]Institute of Bioengineering, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland, [5]Dept of Animal Biotechnology, Kangwon National University, Seoul, Korea, Republic of

We have attempted to employ ovarian preantral follicle as an alternative souce of stem cells and this study was subsequently designed to optimize physical property of a three-dimensional (3D) culture system for the culture of mouse ovarian follicles. To create 3D environment, poly(ethylene) glycol (PEG)-based hydrogel was employed and the preantral follicles retrieved from 2-week-old B6CBAF1 prepubertal mice were cultured in the hydrogel consisting of vinylsulfone-functionalized, 4-arm PEG conjugated with a crosslinker of matrix metalloproteinase (MMP)-sensitive peptide. The structure of crosslinker was Ac-GCRD-GPQG↓IWGQ-DRCG-NH_2 of thiol side chain and flanking cysteine (C) residues form the crosslinking sites. The swelling ratio was determined as the mass ratio of network at the equilibrium of swelling in the dry state, which represents the softness of hydrogel. When the softness was modified within the range of 25.7 down to 15.5 by increasing PEG concentration from 5 to 15% (w/v), it did not influence follicular growth to form pseudoantrum (60-80%; p=0.7646). However, decrease of the softness as low as 20.6 significantly (model effect=0.014) inhibited oocyte maturation (68-70% to 38-46%). The largest number of oocytes formed pronucleus (92 vs. 20-80%, *p*=0.015) and blastocoele (3 vs. 0 oocytes, *p*=0.038) was detected at the swelling ratio of 20.6. No significant difference in the number of oocytes collected (35 to 46%, *p*=0.67) and the number of activated oocytes cleaved after activation (80 to 100%, *p*=0.35) was detected among the treatments. From these results, the PEG-based hydrogel forming a swelling ratio of 20.6 was the optimal for acquiring follicle-derived, developmentally competent oocytes and as a conclusion, hydrogel softness was one of the essential factors for supporting in vitro-growth of preantral follicles under a hydrogel-based, 3D microenvironment.

F-2268

DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO NEURAL PRECURSOR CELLS AND THEIR GLIAL PROGENY FOR ENHANCED TISSUE REGENERATION AFTER SPINAL CORD INJURY

Tobias Fuehrmann[1], Roger Y. Tam[1], Brenda L.K. Coles[1], Derek van der Kooy[1], Cindi Morshead[1], Andras Nagy[2], Molly S. Shoichet[1]

[1]Donnelly Centre for Cellular & Biomolecular Research, University of Toronto, Toronto, ON, Canada, [2]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada

Stem cells hold the promise to provide suitable cell populations to regenerate diseased or injured tissue which the body is unable to heal itself. This includes, but is not restricted to, lesions of the central nervous system. The loss of function after traumatic spinal cord injury (SCI) is due to both the primary mechanical injury and the secondary injury, which results in a cascade of cellular and molecular events, leading to axonal degeneration, demyelination of spared axons, inflammation, axon-growth inhibitory/repulsive molecules at and around the lesion site, and glial scarring. Cell transplantation-based treatment strategies positively influence a number of these targets and transplantation of embryonic- or foetus-derived neural stem/progenitor cells (NPC) into different experimental models of SCI has generally resulted in some degree of functional improvement that was associated with an uncontrolled differentiation of donor cells. Furthermore, transplantation of NPC-derived central glia (i.e oligodendrocytes and astrocytes) has been shown to be more efficient than grafting non-differentiated or uncommitted cells; however, the survival of transplanted cells is limited. Cell-delivery systems that enhance the survival and integration of grafted cells should lead to greater tissue and functional repair.
In the absence of external signals, mouse and human embryonic and iPS cells default to a neural lineage. We characterised (by the expression of SSEA4, Nanog and Oct4) and cultured human iPS in serum-free media, where they rapidly acquired a NPC identity as observed by sphere formation and the expression of neural precursor markers Nestin, SOX2 and Musashi. Upon growth factor withdrawal these cells differentiate into neurons, astrocytes and oligodendrocytes as demonstrated by the expression of microtubule-associated protein 2a+b (MAP2a+b), glial fibrillary acidic protein (GFAP) and O4, respectively. To enhance and direct the differentiation into astrocytes and oligodendrocytes neurospheres were treated with BMP4, CNTF, PDGF and T3 for 3 weeks. Upon plating onto fibronectin coated slides CNTF, PDGF and T3 treated cells appeared small, with few, fine processes. In stark contrast BMP4-derived cells were larger and demonstrated a polygonal morphology, resembling an astrocyte type I phenotype. Longer time points are being investigated to further enrich the cultures for oligodendrocytes and astrocytes.

Furthermore, cells and spheres can be cultured in hydrogels comprised of hyaluronan and methyl cellulose (HAMC) *in vitro* and then transplanted *in vivo*. To enhance cell survival, these hydrogels have been chemically modified with cell adhesion peptides derived from fibronectin and laminin (i.e. RGD, PHSRN, YIGSR, IKVAV) and survival factors (e.g. interferon-β and ciliary neurotrophic factor).

F-2271

MATRIX ELASTICITY CONTROLS BONE FORMATION BY STEM CELLS DEPLOYED FROM VOID-FORMING HYDROGELS

Nathaniel Huebsch[1], Evi Lippens[1], Kangwon Lee[1], Manav Mehta[1], Christopher Madl[1], Maria M. Xu[1], Xuanhe Zhao[1], Ovijit Chaudhuri[1], Karen Alim[1], Akiko Mammato[2], Donald E. Ingber[1], Georg Duda[3], David J. Mooney[1]

[1]School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, United States, [2]Vascular Biology Program, Departments of Pathology & Surgery, Harvard Medical School, Boston, MA, United States, [3]4.Julius Wolff Institute, Charité – Universitätsmedizin Berlin and Berlin-Brandenburg Center for Regenerative Therapies, Berlin, Germany

Biophysical cues from the cellular micro-environment, including extracellular matrix (ECM) elasticity, have been shown to dramatically influence stem cell fate in vitro, but the influence of those cues on stem cell-mediated tissue regeneration remains unclear. A key challenge hampering in vivo studies linking matrix elasticity to stem cell behavior is a lack of macroporous materials that allow precise control over the molecular cell-material interface under conditions that are amenable to cell encapsulation. To allow analysis of the effects of matrix elasticity on stem cell mediated tissue regeneration, we fabricated cell-encapsulating, “void-forming” hydrogel materials that form pores in situ, after injection, to facilitate cell deployment to injured tissues without affecting the adhesion ligand presentation or mechanical properties of the hydrogel surrounding pores. We applied these materials towards bone regeneration using mesenchymal stem cells (MSC).
Void-forming hydrogels were fabricated by co-encapsulating cells along with rapidly degrading, gel-based porogens (typically 150µm diameter) into a more slowly degrading “bulk” hydrogel network. Sacrificial porogens were formed using oxidized alginate that exhibits rapid hydrolytic degradation, which was subsequently extruded under co-axial airflow into a calcium chloride bath. The bulk phase was formed from a high molecular weight alginate polymer modified with integrin-binding RGD peptides. Void density and formation were verified by scanning electron microscopy and mechanical testing. By modulating the extent of crosslinking and succeptibility to hydrolytic degradation of porogens, it was possible to vary the initiation of MSC release from 5-50 days in vitro or 5-20 days in vivo. Further, in cranial defect studies, human MSC (hMSC) deployed from void-forming hydrogels led to more extensive bone regeneration than hMSC deployed either in standard, non-degrading hydrogel materials or saline.
To study the effects of matrix elasticity on hMSC-mediated bone regeneration, we first performed in vitro studies to confirm that modulating the rigidity of the “bulk” component of gels surrounding voids could regulate MSC behaviors. These studies revealed a strong role for matrix elasticity, with MSC proliferation and osteogenic commitment being optimal at an intermediate range (20-60 kPa). Strikingly, in cranial defect studies, bone regeneration by hMSC delivered in void-forming hydrogels depended strongly on matrix elasticity, with regeneration occurring optimally when cells were deployed from matrices of intermediate elastic modulus (60 kPa versus 5 or 110 kPa). This verifies that matrix elasticity can directly control cell behaviors in vivo. More broadly, this strategy for fabricating void-forming materials may be useful for studying how specific cues from biomaterials, in combination with cues from host tissues, regulate transplanted stem cell phenotype. These materials could be modified with soluble cues to fine-tune control over stem cell behavior - or to probe the molecular mechanisms through which host micro-environments alter stem cells.

F-2272

SURFACE MODIFICATION OF POLYURETHANE AND POLY(SULFONE) FILMS BY UV- ASSISTED TREATMENT FOR WETTABILITY AND CELL SPREADING ENHANCEMENT FOR USE IN TISSUE ENGINEERING

Felipe Kessler[1], Daniela Steffens[2], Gabriela A. Lando[1], **Patricia Pranke**[3], Daniel E. Weibel[1]

[1]*Laboratory of Photochemistry and Surfaces (LAFOS), Institute of Chemistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil,* [2]*Hematology and Stem Cell Laboratory, Faculty of Pharmacy, Federal University of Rio Grande do Sul, Porto Alegre, Brazil,* [3]*Hematology and Stem Cell Laboratory, Faculty of Pharmacy, Federal University of Rio Grande do Sul; Stem Cell Research Institute, Porto Alegre, Brazil*

The relationship between the surface chemistry of materials and cellular response has great importance for the application of biomedical materials, regenerative medicine and tissue engineering. Because it is the surface of the biomaterial which first comes into contact with the body when the biomaterial is used as a medical device, the initial response of the living body to it must depend on its surface properties. Poly(sulfone) (PSU) and Polyurethane (PU) have many applications in medical devices but pristine PSU and PU are slightly hydrophobic polymers showing WCA of about 81o and 85o respectively. For this reason, the surface PSU and PU films were modified with ultraviolet (UV) light in the presence of oxygen to improve their wettability, adhesion and cell spreading properties for 30 (PSU30 and PU30), 60 (PSU60 and PU60) and 120 (PSU120 and PU120) minutes. To evaluate the results of the surface treatments, analysis of the grafted functional groups was accessed by X-ray photoelectron spectroscopy (XPS) and the wettability evaluated by water contact angle (WCA). The untreated and treated films were then verified for biological properties. Dental pulp mesenchymal stem cells were seeded on the biomaterials and evaluated for adhesion and viability for 1, 4 and 7 days. UV irradiation of the films in the presence of oxygen, decreased the WCA to less than 30o within 90 min of photolysis for both the PSU and PU films. The XPS analysis showed that the surface of the PSU and PU were oxidized into oxygen functional groups, containing C-O (in increasing amounts), C=O, and COO components, making the PSU and PU surface hydrophilic. Comparing the number of adhered cells in the control group to all the other groups, PSU30 ($p=0.947$), PSU120 ($p=0.189$) and PU120 ($p=1$) showed no statistical difference. Among the groups where the films were made with PSU, there is no statistical difference in terms of adhesion of cells ($p>0.6$ for all comparisons). In PU films, cells adhere statistically more on films with 120 min treatment than PU0 and PU30 ($p<0.001$). By the results obtained it is possible to observe that the treatment influenced the adhesion of cells, as the films exposed to UV light with oxygen showed a larger number of adhered cells compared to the untreated films, although within each group this statistical difference is not present (with the exception of PU120). The number of adhered cells in the treated films was comparable to the number of adhered cells in the control group. Concerning the viability assay, with the exception of day 4, there was no statistical difference among all the groups and the control. It is believed that this behavior could be attributed to the hydrophilicity of the films, as all of them had low contact angle with water for cells. It was also expected that the cells would proliferate more on more hydrophilic surfaces, which did not occur to the PSU groups on day 7 of analysis. An explanation for the different behavior between the PSU and PU films could be attributed to the findings of some studies that have demonstrated that cells adhere and proliferate more on substrates which contain N-H groups in their structures. The obtained data also showed that the cell response not only depends on the hydrophilicity of the materials but on the chemical surface alterations which occur as a result of UV-assisted treatment in the presence of oxygen. Better cell adhesion, spreading and growing on the PSU and PU substrates modified by the present UV methodology confirmed the biocompatibility of the treated surfaces.

F-2273

SEEDING OF A NOVEL BIODEGRADABLE NANOCOMPOSITE ELASTOMERIC POLYMER WITH MYOBLASTS AND ENDOTHELIAL CELLS FOR THE DEVELOPMENT OF A TISSUE ENGINEERED VASCULAR GRAFT

Ryan J. Dee[1], Luca Urbani[2], Panagiotis Maghsoudlou[2], Paolo De Coppi[2], Alexander Seifalian[3]

[A]Centre for Mathematics and Physics in the Life Sciences and Experimental Biology, [B]Paediatric Surgery Unit, Institute of Child Health, [C]Centre for Nanotechnology and Regenerative Medicine, Royal Free Hospital, [1]University College London, London, United Kingdom

Introduction: Congenital Heart Defects (CHDs) are the most common congenital malformations in the UK, with severe cases leading to 70% mortality in the first year if left untreated. Current approaches use vascular grafts when redesigning the vascular network. However, many of these lack growth potential, leading to either delayed surgery or multiple operations, along with complications such as thrombosis and calcification. A tissue engineered approach may be beneficial in cases of pediatric CHD. Our aim is to produce a tissue engineered vascular graft with a novel synthetic scaffold designed to have the mechanical properties of native vessels and optimised for the attachment of muscle and endothelial cells.

Methods: The polymer, a novel nanocomposite based on polycaprolactone, was fabricated by casting at 65°C and by phase inversion in reverse osmosis water to create a film and a porous foam respectively. Characterisation of the surface chemistry, mechanical properties and wettability was undertaken for both fabrication methods by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, tensile testing and contact angle measurements. The porous polymer was also imaged using a fluorescent dye to determine surface pore dimensions and frequency.

Human umbilical vein endothelial cells (HUVEC) were freshly isolated from human umbilical cord and cultured on fibronectin in EGM^{TM}-2 until passage 2 and seeded on to cast and porous polymer disks at a density of 1.25×10^5 cells/cm^2. C2C12 myo-

blasts were seeded at 6.25×10^4 cells/cm^2. Attachment of cells to the polymer was assessed by scanning electron microscopy (SEM) and histological analysis at 2, 4 and 7 days.
Results: ATR-FTIR spectroscopy showed a quantitative difference in several peaks between the two fabrication methods, indicating that fabrication method has an effect on which moieties are most likely to migrate to the material's surface. Cast polymer was significantly stronger and stiffer than porous polymer, with the porous polymer more closely resembling a native vessel in its stiffness than the cast. Cast polymer had a significantly larger contact angle than the porous polymer, due the presence of pores on the porous polymer's surface, which were also seen in fluorescence imaging.
SEM of seeded polymer showed cell attachment of both cell types on both cast and porous polymer disks. HUVEC had a flat morphology whereas C2C12 formed elongated myotube-like structures. The greatest number of cells were seen at day 7.
Conclusion: For the first time we have shown that a novel nanocomposite polymer can support the growth of two cell types necessary for a functional vessel. This is an important first step in our goal to create a tissue engineered vascular graft.

F-2274

THE POTENTIAL TO ENHANCE STEM CELL SELECTION THROUGH A PHOTO-CONVERTING REPORTER GENE

A.J. Mellott[1], Heather Shinogle[2], David Moore[2], Michael Detamore[1]
[A]Chemical and Petroleum Engineering, [1]University of Kansas, Lawrence, KS, United States

When transfecting stem cells with fluorescent reporter genes, the low signal to noise ratio can impede the efficient isolation of positively transfected stem cells for therapeutic treatments in tissue engineering and other regenerative medicine applications. By exploiting an irreversible photo-convertible (green to red) reporter gene, we have demonstrated that it is possible to increase the signal-to-noise ratio of positively transfected human umbilical cord mesencymal stem cells (hUCMSCs) and identify target hUCMSCs for isolation.
We isolated hUCMSCs from human umbilical cords according to protocols approved by the University of Kansas Institutional Review Board. hUCMSCs were expanded to passage 2 (P2), then frozen and stored. Frozen hUCMSCs were thawed, and expanded to P5. At P5, hUCMSCs were treated with 10 µM of Y-27632 Rho-associated protein kinase (ROCK) inhibitor (Reagents Direct) for 1 hour prior to transfection. hUCMSCs were transfected with Dendra2 (Clonetech) via Nucleofection™ (Lonza), and immediately plated in hUCMSC media treated with 10 µM of Y-27632 ROCK inhibitor. 24 hours after transfection, hUCMSCs were analyzed for cell viability using SYTOX RED (Life Technologies), transfection efficiency, and photo-conversion efficiency via an Olympus spinning disc confocal microscope. Additionally, a subset of transfected hUCMSCs were “flash-sorted” onto an 8-well chamber slide (IBIDI,) using a Beckman Coulter MoFlo XDF fluorescent activated cell sorter (FACS) to isolate positively transfected hUCMSCs that had undergone photo-conversion after passing through a 355 nm laser while in the flow stream 24 hours after transfection. After hUCMSCs were “flash-sorted”, hUCMSCs were immediately imaged through confocal microscopy to confirm accurate sorting and photo-conversion.
We found 5 µg of plasmid Dendra2 per 500,000 cells was an effective transfection concentration for producing reproducible results. 24 hours after transfection, we exposed a sub-set of transfected hUCMSCs to UV light at a frequency of 1 Hz, and found that green fluorescence continually decreased as red fluorescence continually increased. After exposure to UV light at 1 Hz for 10 minutes, all hUCMSCs in the field of view fully converted. Furthermore, we demonstrated in a time-lapse video that we confined a UV light beam onto two single cells, and were able to fully photo-convert the two exposed hUCMSCs, without photo-converting surrounding hUCMSCs. Lastly, we ran positively transfected hUCMSCs through the FACS, and photo-converted the hUCMSCs while in the flow stream. Fully photo-converted cells were sorted into a single well of an 8-well chamber slide, and immediately imaged on our confocal microscope. We successfully demonstrated that it was possible to “flash-sort” hUCMSCs that have undergone photo-conversion in the flow stream. However, while the technique has been demonstrated, further research is needed to further develop the technique to increase the efficiency and accuracy of sorting photo-converted cells.

We conclude that we were able to accurately identify positively transfected hUCMSCs by increasing signal-to-noise ratio through the use of an irreversible photo-converting reporter gene. Furthermore, we have shown that it is possible to photo-convert positively transfected hUCMSCs in the flow stream of a FACS and successfully isolate photo-converted cells from non-converted cells, but follow-up work is needed to further develop and refine the technique.

F-2275

FABRICATION AND CHARACTERIZATION OF CONDUCTIVE GRAPHENE SUBSTRATE FOR EVALUATING THE BEHAVIOR OF NEURAL STEM CELLS

Sasan Jalili Firoozinezhad, Mohamadhasan Mohamadzadeh Moghadam, Ebrahim Shahbazi, Hossein Baharvand
Stem Cells and Developmental Biology, Royan Institute, Tehran, Iran, Islamic Republic of

Introduction
Controlling the microenvironmental factors via physical, chemical and electrical properties of artificial substrates is one of the main goals of tissue engineering. Various nanomaterials with unique electrical properties, such as carbon nanotubes (CNT) or conductive polymers, have been studied as substrates or scaffolds for stem cells. Graphene, a novel one atom thick sheet of carbon atoms arranged in 2D honeycomb structure, due to its great structural and electrical properties is attracting a great deal of attention as a potential material to be significantly useful in tissue engineering applications.

Materials and methods
Graphene has been synthesized by modified Hummer's method. To do so, graphite powder was stirred in concentrated sulfuric acid medium for couple of hours. Subsequently oxidizing agent, potassium permanganate, was added gradually to the dispersion. In order to completing the oxidation reaction, the dispersion was left overnight. Then, hydrogen peroxide was added to terminate oxidation reaction. In this step the product was mainly graphite oxide. After washing with dionized water and hydrochloric acid solution, resultant was sonicated to reach single layer of graphene oxide (GO). Afterward by applying hydrazine monohydrate the graphene (G) sheet was achieved. Scanning Electron Microscopy (SEM) was used to evaluate the morphology of G and GO. The thickness of single layer GO was measured with Atomic Force Microscopy (AFM) and X-ray Diffraction (XRD) assessments used to identify the structure of G and GO. The conductivity of G film was measured by 4-point probe resistivity test. To study the size of GO sheets and investigating their surface wrinkling, Optical Microscopy was applied.
In the next step, in order to evaluate the biocompatibility of the substrates and proliferation of neural stem cells derived from human ploripotent stem cells (hPSCs), MTS assay was performed in 3, 7 and 14 days.

Results and discussion:
SEM images of G and GO sheets revealed uniform coverage of thin sheets on the surface and thin wrinkled paper-like structure of G. The layers have been exfoliated efficiently to ultrathin sheets with wavy structures. The AFM data showed that the GO has a thickness of about 0.7 nm which is in accordant with other previous studies.
The XRD pattern of GO exhibits a characteristic peak at about 11° which is corresponding to the (0 0 1) diffraction peak of graphene oxide. After reduction, the (0 0 1) peak of GO disappears and an additional peak observed around 26° which is the characteristic peak of graphene.
Owing to optical microscopy images, the average size of GO sheets was about 100 µm which is very suitable to achieve high electrical conductivity.
The 4-probe resistivity test showed that the G substrate has connectivity about 3.9 S/cm which is useful for the intended purpose of the current research.
The MTS assay also performed to confirm the cell viability data. There was no difference ($p > 0.05$) between Tissue Culture Polystyrene (TCPS), GO and G substrates, demonstrating that G and GO are not toxic for the cells. The proliferation of the cells also promoted on G substrates.

Conclusion
Electrical conductivity, like cocktails of growth factors and substrate properties, is able to stimulate cell growth and differentiation. The electrical conductive graphene substrate allows for proliferation and differentiation of neural stem cells and hold great promise for neural tissue engineering applications.

F-2276

BIOENGINEERED HUMAN NEOPAPILLAE FOR TRANSPLANTATION AND HAIR FOLLICLE EQUIVALENTS FOR SUBSTANCE TESTING

Beren Atac[1], Reyk Horland[1], Alexander Thomas[1], Ilka Wagner[1], Roland Lauster[1], Uwe Marx[2], Gerd Lindner[1]
[1]Medical Biotechnology, Technical University of Berlin, Berlin, Germany, [2]TissUse GmbH, Spreenhagen, Germany

In this study we provide a promising approach to produce tissue engineered dermal papilla equivalents for hair transplantation in vivo and hair follicle equivalents for substance testing in vitro. Replacing lost hair due to pattern baldness or chemotherapy remains a significant unmet medical need. On the other hand, in vitro micro hair follicle equivalents provide a platform for investigating hair follicle biology and substance testing concerning skin and hair related pharmaceuticals. The dermal papilla is discussed as mesenchymal stem cell niche and command center of the hair follicle. We will describe neopapillae formation after the expansion of human adult dermal papilla cells (DPCs) in vitro and how this process is optimised for transplantation purposes considering pre-clinical requirements. Expanded co-culture of mesenchymal originated neopapillae with epithelial keratinocytes, neuro-ectodermal melanocytes and mesodermal derived endothelial cells reconstitute fibre producing microfollicles. The adjustment of this bioengineering process for reproducibility and the simulation the physiological hair follicle environment will be covered. At last we will discuss current status of the pre-clinical tests and capabilities of the hair follicle equivalent with challenges and opportunities we are facing.

F-2277

POLY-L-LYSINE INCREASES THE EX VIVO EXPANSION AND ERYTHROID DIFFERENTIATION OF HUMAN HEMATOPOIETIC STEM CELLS, AS WELL AS ERYTHROID ENUCLEATION EFFICACY

Kwang-Sook Park[1], Jongchan Ahn[1], Hyun Ok Kim[2], Soo-Hong Lee[1]
[1]CHA Univ, Seongnam-si, Korea, Republic of, [2]Laboratory Medicine, Yonsei Univ, Seoul, Korea, Republic of

Hematopoietic stem cells (HSCs) are continuously stimulated by physical interactions with bone marrow or umbilical cord niches, and chemical factors found within these niches. The interactions between HSCs and niche-derived extracellular matrix (ECM) molecules play an important role in *ex vivo* expansion and differentiation of HSCs. The ECM within the niche can be mimicked by modification of the cytokine composition, elasticity, topography, and/or charge of various cell culture substrates. This work employed cell culture plates coated with several concentrations of poly-l-lysine (PLL), a positively charged synthetic amino acid chain. Culture substrates that employed relatively high initial coating concentrations of PLL (0.01, 0.1 w%) significantly increased the total number of HSCs during *ex vivo* expansion of CD34+ cells isolated from human umbilical cord blood, as well as erythroid differentiation; however, the percentage of HSCs with CD34+Lin− surface expression remained unaltered. Furthermore, the 0.01% PLL substrate stimulated enucleation of erythroid cells, leaving behind a number of extruded nuclei on the bottom of the culture plate, followed by an increase in the number of erythrocytes. Thus, PLL will likely prove useful to enhance the expansion of HSCs and erythroid cells, in addition to the generation of red blood cells (RBCs).

F-2278

ENGINEERING A THREE-DIMENSIONAL MODEL OF THE DEVELOPING HUMAN HEART FOR IN VITRO DRUG TESTING

Petra Kerscher, Alexander Hodge, Blakely Bussie, Elizabeth Lipke
Chemical Engineering, Auburn University, Auburn, AL, United States

Annually, about 25,000 newborns in the United States are born with a congenital heart defect; however, the causes of congenital heart defects are only partially understood, particularly in the absence of genetic mutations. In this study, we use human induced pluripotent stem cells (hiPSCs) to create *in vitro* model for investigating the effects

of known and potential cardiac mutagens during stem cell differentiation. To better mimic *in vivo* conditions, our *in vitro* model system was created by encapsulating the hiPSCs in a degradable hydrogel material, poly(ethylene glycol) PEG-fibrinogen, to form three-dimensional (3D) developing tissue constructs. This 3D *in vitro* tissue model facilitates more complex and physiologically relevant cell-cell and cell-matrix interactions than two-dimensional (2D) cell culture, which has been traditionally employed for investigating the causes of congenital heart defects *in vitro*, while allowing for higher throughput experiments during drug testing than would be possible using animal models. IMR 90 hiPSCs (Clone 1) were cultured and expanded on Matrigel using mTeSR-1 media. HiPSCs were passaged using Versene (EDTA). To validate the feasibility of this system for identifying chemicals that cause congenital heart defects, hiPSCs and hiPSC-derived cardiomyocytes were exposed to thalidomide, a well-known mutagen which caused birth defects, including congenital heart defects, in thousands of newborns in the 1950s. Cell viability and proliferation were quantified at concentrations ranging from 1 to 70 μM thalidomide; mesoderm formation is known to be affected by thalidomide within this range. Cell viability was visualized using the live/dead assay (Calcein AM, Ethidium Homodimer) and proliferating cells were identified through immunocytochemistry to detect proliferating cell nuclear antigen (PCNA). No significant difference in cell number or viability was found compared to the drug carrier alone, indicating that thalidomide is not inducing a significant change in cell number at these concentrations and facilitating future studies to investigate other mechanisms involved in causing the known developmental mutations. Using a conventional cardiac differentiation method, thalidomide was also introduced at early time points of stem cell differentiation (days 0, 5, and 10) to determine changes in mesoderm formation. To form the 3D developing tissue constructs, first mouse embryonic stem cells (mESCs) and then hiPSCs were mixed with the aqueous PEG-fibrinogen hydrogel precursor including the photoinitator Eosin Y, N-vinylpyrrolidone (NVP), and triethylamine (TEOA) and crosslinked by exposure to light. Proliferation and differentiation of mESCs into contracting stem cell derived-cardiomyocytes was supported within the PEG-fibrinogen tissue constructs. Based on these successful experiments, hiPSCs were encapsulated within the PEG-fibrinogen tissue constructs and shown to remain viable. This study will be followed by initiating hiPSC differentiation within PEG-fibriongen. HiPSC differentiation within the 3D hydrogel will provide insight in the interactions of thalidomide with the developing human heart and enhance our overall understanding of human heart development.

F-2281

BIOACTIVITY EVALUATION BONE LIKE METAL ION DOPED HYDROXYAPATITE NANOPOWDERS

R. Beklem Bostancioglu[1], Mevlüt Gurbuz[2], Aydin Dogan[2], A. Tansu Koparal[1]
[A]Biology, [B]Material Science and Engineering, [1]Anadolu University Graduate School of Sciences, Eskisehir, Turkey

Differentiation of mesenchymal stem cells (MSCs) to special cell types and biomaterial engineering are two disciplines of tissue engineering and regenerative medicine that have been developing. The field of regenerative medicine deals with repair, replaces, and regenerates tissues or organs that are damaged by injury or disease. The tissue engineering predominantly involves recreating the ECM environment to govern the cell behavior on a desired scaffold with the aim of implantation at the target site. The suitable implant that will change the solid tissue should have bioactive potential and connected a chemical link between bone and implant surface. In this mention, materials are that formed glasses, ceramics, glass-ceramic complexes and composites are bioactive materials. Before come onto the market of new biomaterials, in order to understand whether caused there is an unwanted effect in the body, after subjected to in vitro, and then in vivo tests, carried out clinical trials to determined biological safety and performance.
This study reports on in vitro evaluation of new synthesized ceramic and metal ion doped hydroxyapatite nanopowders (HAP-Ag/Cu and Zn/Ag). The effects of bone like metal ion doped hydroxyapatite nanopowders on adhesion, proliferation and differentiation of osteoblast cells in vitro measured. MTT cytotoxicity test and adhesion test show that HAP-Ag/Cu and Zn/Ag have good biocompatibility than pure HAP and TCP (tricalciumphosphate) and promote cell viability and proliferation by ratio of more than 50% compared to the HAP and TCP on human adipose derived human mesenchymal stem cells (hMSC). HAP-Ag/Cu surfaces lead to good cellular adherence and show the highest viable cell densities after 10 days while HAP and TCP surfaces exhibit limited cellular growth. We applied to alizarin red staining for detect to osteoblast differentiation. The results confirm that nanopowders are biocompat-

ible and have no negative effects on the hMSCs both proliferation and osteoblastic differentiation in vitro. Therefore, the HAP-Ag/Cu and Zn/Ag composite nanopowders may have good prospect in future bone tissue engineering applications.

F-2282

HUMAN EMBRYONIC STEM CELL-DERIVED TISSUE-ENGINEERED CARDIAC PATCH WITH ADVANCED STRUCTURE AND FUNCTION

Donghui Zhang[1], Ilya Shadrin[1], Jason Lam[2], Haiqing Xian[2], Ralph Snodgrass[2], Nenad Bursac[1]
[1]Biomedical engineering, Duke University, Durham, NC, United States, [2]VistaGen Therapeutics, Inc., San Francisco, CA, United States

Human embryonic stem cell-derived cardiomyocytes (hESC-CMs) provide a promising source for cell therapy and drug screening. Several high-yield protocols exist for hESC-CM production; however, methods to significantly advance hESC-CM maturation are still lacking. At the macroscopic level, the inadequate CM maturation compromises the tissue-level functions yielding slower action potential conduction and lower contractile stresses. Building on our previous experience with mouse ESC-CMs, we set out to explore the effects of 3-dimensional (3D) tissue-engineered culture environment and cardiomyocyte purity on structural and functional maturation of hESC-CMs. To this end, we generated 2D monolayers and 3D cardiac patches using varying percentages of human ESC-derived cardiomyocytes, purified using antibodies against cardiac-specific surface marker SIRPA (CD172a). hESC-CMs within cardiac patches were aligned uniformly by locally controlling the direction of passive tension. Relative to 2D monolayers, hESC-CMs in 3D patches exhibited significantly higher conduction velocities (CVs), longer sarcomeres (2.09±0.02 vs. 1.77±0.01 μm), and enhanced expression of genes involved in cardiac contractile function, including cTnT, α-MHC, CASQ2 and SERCA2. The CVs in cardiac patches increased with cardiomyocyte purity, reaching 21.2±1.5 cm/s in patches containing 80-90% hESC-CMs, 4-fold higher than previously measured in engineered human heart tissues. More importantly, force testing of cardiac patches revealed contractile forces and active stresses of 3.0±1.1 mN and 11.8±4.5 mN/mm2, respectively, 2.2-150 fold higher than previously reported for in vitro engineered myocardium, and nearing levels of native human myocardium. Additionally, these 2 week-old patches demonstrated significant positive inotropy with isoproterenol administration (1.7±0.3-fold force increase, EC50 = 95.1 nM), indicating a functional sarcoplasmic reticulum characteristic of mature Ca2+-handling.
Together, our results show successful creation of highly functional 3D cardiac tissues made of hESC-derived cardiomyocytes. Furthermore, we demonstrate that relative to standard 2D culture, 3D patch environment significantly enhances certain aspects of hESC-CM maturation. The electromechanical properties measured in our cardiac patches surpass those of all other in vitro engineered human myocardium previously reported and, to our knowledge, provide currently the closest functional approximation of native human myocardium. We believe that the use of non-genetic methods to purify CMs along with the achieved level of advanced maturation warrant the use of this near-physiological tissue-engineering platform for future drug and toxicology studies and novel cell-based cardiac therapies.

F-2283

EFFECTS OF FERULIC ACID-LOADED HYDROGEL ON CISD2-DEFICIENT MURINE INDUCED PLURIPOTENT STEM CELL UNDER HYDROGEN PEROXIDE-INDUCED OXIDATIVE STRESS

Yung-Hsin Cheng[1], Yun-Ching Chang[2], Hsin-Yu Kuo[2], Chia-Jung Lin[2], Ming-Long Tsai[1], Shih-Chieh Chou[2], Shih-Hwa Chiou[1]
[1]Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan, [2]National Yang-Ming University, Taipei, Taiwan

CISD2(synonyms ZCD2, Noxp70, Miner1, ERIS), a member of CDGSH iron sulfur domain protein family, is a mitochondria outer membrane protein. Recent studies have demonstrated that CISD2 deficiency causes mitochondria

dysfunction and leads to cell death. Mitochondrial dysfunction may influence multiple signaling pathways including oxidative stress and apoptosis that is strongly associated with aging-related diseases. The overproduction of reactive oxygen species (ROS) leads to oxidative stress is commonly associated with apoptosis and senescence that plays a key role in aging. Ferulic acid (4-hydroxy-3-methoxy cinnamic acid) (FA) is an excellent antioxidant and relatively stable in air. FA has been proven to have ability to prevent ROS-induced diseases. In the previous study, we developed thermosensitive chitosan/gelatin/glycerol phosphate (C/G/GP) hydrogel which is liquid at room temperature but gels at 37°C under neutral pH. The object of the study was aimed to evaluate the possible protective effects of FA on hydrogen peroxide (H_2O_2)-induced oxidative stress $CISD2^{-/-}$ miPSCs and the feasibility of use the thermosensitive FA-loaded C/G/GP hydrogel for tissue engineering application.
In the study, we established the $CISD2^{+/+, -/-}$ murine induced pluripotent
stem cells (miPSCs) from murine fibroblasts by retroviral introduction of
Oct3/4, Sox2, c-Myc and Klf4. $CISD2^{+/+, -/-}$ miPSCs were positive for alkaline phosphatase, SSEA1 and Oct4. The mitochondrial degeneration was observed in the $CISD2^{-/-}$ miPSCs under the transmission electron microscope. The results of lactate dehydrogenase assay showed that $CISD2^{-/-}$ miPSCs was more sensitive to H_2O_2-induced oxidative stress compared with $CISD2^{+/+}$ miPSCs. The results demonstrated that 1000 μM of FA might be the threshold to treat $CISD2^{-/-}$ miPSCs without cytotoxicity. The results of chemiluminescence assay showed that FA-loaded C/G/GP hydrogel could significantly decrease the oxidative stress induced by H_2O_2. From the results of calcein acetoxymethyl ester stain and cell proliferation assays, $CISD2^{+/+, -/-}$ miPSCs cultured in FA-loaded C/G/GP hydrogel had normal cell viability and cell proliferation that indicated the hydrogel was non-cytotoxic. The results of teratoma assay showed that $CISD2^{-/-}$ miPSCs retained their pluripotency when cultured within FA-loaded C/G/GP hydrogel. From the results of the study, the combination of miPSCs and thermosensitive FA-loaded C/G/GP hydrogel may treat cells from the damage caused by oxidative stress and may have potential applications in tissue engineering.

F-2284

ENGINEERING BONE GRAFTS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Giuseppe Maria de Peppo[1], Ivan Marcos Campos[2], Dave Kahler[1], Dana Alsalman[1], Linshan Shang[1], Gordana Vunjak-Novakovic[2], Darja Marolt[1]

[1]The New York Stem Cell Foundation, New York, NY, United States, [2]Department of Biomedical Engineering, Columbia University, New York, NY, United States

INTRODUCTION: Integrity and functionality of the skeletal system can be affected by
congenital defects, trauma and disease, and the implantation of bone grafts is required in a large number of clinical cases. Current treatments have limited clinical potential and new therapeutic approaches are needed to develop effective therapies for complex bone reconstructions. Human induced pluripotent stem cells (iPSC) display high regenerative properties and offer unique opportunities to grow unlimited amounts of autologous bone grafts tailored to the patient and the defect being treated. The objectives of this study were to 1) engineer patient-specific bone grafts using human iPSC derived from different tissues by implementing different reprogramming strategies and the osteoconductive scaffold - perfusion bioreactor culture model, 2) explore the molecular changes associated with culture in perfusion bioreactor, and 3) assess phenotypic stability of engineered tissues after in vivo implantation. MATERIALS AND METHODS: Human iPSC lines were generated from dermal fibroblasts and bone marrow stromal cells using retroviral vectors, Sendai virus and episomal vectors. Human embryonic stem cell line H9 was used as control for all experiments. iPSC lines were karyotyped, characterized for pluripotency and induced into the mesenchymal lineage for 7 days. Mesenchymal progenitors were expanded, characterized by probing surface marker expression and differentiation potential toward the osteogenic, chondrogenic and adipogenic lineages in monolayer and pellet cultures, and cultured on decellularized bovine scaffolds (4 mm Ø x 4 mm height) under constant flow perfusion for 5 weeks (linear flow velocity of 800 μm/s). Global gene expression profiles were evaluated by microarray analysis prior and after culture in bioreactors. Bone development was investigated by biochemical, histological

and immunohistochemical analyses, and μCT imaging over the bioreactor culture and after 12-week subcutaneous implantation in mice. RESULTS: Differences in surface antigen and global gene expression profiles of mesenchymal progenitors derived from different iPSC lines corresponded to their proliferation potential and differentiation abilities toward the osteogenic, chondrogenic and adipogenic lineage. Bioreactor cultivation resulted in repression of genes involved in proliferation and tumorigenesis, and upregulation of genes associated with osteogenesis and bone development, and the formation of mature bone tissue that displayed stable phenotype after 12-week implantation in vivo. CONCLUSION: Our biomimetic strategy opens the possibility to construct patient-specific bone grafts for personalized applications, and generate qualified experimental models to study bone biology under normal and pathological conditions, as well as test new drugs using selected pools of human iPSC lines.

F-2285

AUTOMATED ANALYSIS OF CONTRACTILITY IN THREE-DIMENSIONAL, FORCE GENERATING HUMAN ENGINEERED HEART TISSUE: TECHNOLOGY AND POTENTIAL APPLICATIONS

Sebastian Schaaf[1], David Letuffe-Breniere[1], Kaja Breckwoldt[1], Thomas Schulze[1], Aya Shibamiya[1], Marco Mewe[2], Alexandra Eder[1], Andrea Stöhr[1], Marc Hirt[1], Thomas Eschenhagen[1], Arne Hansen[1]

[A]Department of Experimental Pharmacology and Toxicology, [B]Department of Pharmacology for Pharmacists, [1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Human pluripotent stem cell-derived cardiomyocytes show poor sarcomeric structures and cellular orientation in 2D cell culture conditions, and techniques to study physiological parameters are limited. This study analyzed the use of human pluripotent stem cell-derived cardiomyocytes in engineered heart tissues (EHT) to detect pro-arrhythmic drug effects based on video-optical analysis of contractile parameters. Methods and results: Cardiomyocyte differentiation of human pluripotent cells (embryonic stem cells and induced pluripotent stem cells) was achieved by a growth factor-based three stage protocol. Differentiated cardiomyocytes displayed immaturity with poorly organized sarcomeric structure and random orientation. To promote maturation EBs were dissociated into single cells and fibrin-based EHTs were generated. In this format human pluripotent stem cell-derived cardiomyocytes developed a high degree of differentiation, sarcomeric organization and alignment. Furthermore, human EHTs displayed a regular beating pattern for several weeks. Electrophysiological characterization indicated that EHT-derived cardiomyocytes have action potential durations similar to adult cardiomyocytes but upstroke velocities and maximal diastolic potentials of immature cardiomyocytes. Pharmacological characteristics and responses to pro-arrhythmic compounds were evaluated by automated video-optical recording and analysis of spontaneously beating human EHTs. These experiments revealed that pro-arrhythmic substances led to reproducible and concentration-dependent alterations of EHT contractions. Substances tested include Lidocain, Ajmalin, Quinidine, Ibutilide and E4031. Threshold values for changes of contractile parameters matched well with findings from ion channel assays, especially hERG channel inhibitors led to characteristic changes of the relaxation phase. No systematic differences were detected between human embryonic stem cell- and human induced pluripotent stem cell-derived cardiomyocytes. Conclusion: Human EHTs are a promising platform for automated toxicology screens and for in vitro experiments on human cardiomyocytes in general.

F-2286

KERATINOCYTE DIFFERENTIATION OF QUALITY AND QUANTITY CULTURED PERIPHERAL BLOOD HUMAN CD34 POSITIVE CELLS

Rica Tanaka[1], Kayo Arita[1], Sachie Jitsukawa[1], Rie Hirano[1], Takayuki Asahara[2], Hiroshi Mizuno[1]

[1]Department of Plastic and Reconstructive Surgery, Juntendo University School of Medicine, Bunkyo-ku, Japan, [2]Department of Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan

Background:

Recently, we have disclosed the newly developed quantity and quality control culture (QQc) system to potentiate the vasculogenic property of EPCs for tissue repair and skin regeneration. Skin regeneration is thought to occur by secondary effect of vasculogenesis but direct differentiation of EPC to keratinocyte is not yet well investigated. QQc CD34+ cells have shown high potential of skin regeneration in murine wound healing model. Herein, we investigate the possibility of direct contact differentiation of QQc EPC to keratinocyte.
Materials and Methods: Peripheral blood (PB) CD34+ cells were isolated as EPCs from female volunteers and cultured in QQc for one week. Neonatal male keratinocyte was purchased and passaged twice before usage. In order to determine keratinocyte differentiation of EPC by direct contact, pre and post QQc EPCs were co-cultured with keratinocyte in keratinocyte medium (KGM) for one week. Pre and post QQc EPCs were confirmed not to express Keratin 14 (K-14) marker prior to co-culture with keratinocyte by FACS analysis and Immunocytochemistry. The PKH26 labeled pre and post QQc EPC were stained for FITC labeled K14 and analyzed for keratinocyte differentiation by FACS and immunocytochemistry. PKH26/FITC K-14 double positive cells were FACS sorted cells for FISH analysis to confirm keratinocyte differentiation.
Result:Expression of PKH26 and K-14 double positive cells for co-culutred post QQc EPC were higher compared to co-cultured pre QQc EPC by FACS analysis (3.28% vs 1.66%) and immunocytochemistry (10% vs 2%). FISH analysis of PKH26 labeled female derived post QQc EPC positive for K-14 demonstrated positive staining for XX choromosomes suggesting direct contact induced differentiation of post QQc EPC to keranitocyte.
Discussion: We have demonstrated the possibility of post QQc EPCs acquiring potential to differentiate into keratinocyte by direct contact of keratinocyte. Our results indicate that QQc not only enhances vasculogenic potential of EPC but enhances skin tissue regeneration by enhancing differentiation potential of its contacted cell type. QQc system maybe novel alternative cell based therapy for tissuse regeneration.

F-2287

EFFECTS OF NANOFIBER-TB4 TRANSFECTED CELL PATCHES ON ACTIVATING EPICARDIAL CELLS AND REGENERATING MYOCARDIUM AFTER MYOCARDIAL INFARCTION

Qiangli Wang, Haijie Wang, Zhihua Li, Yuzhen Tan
Shanghai Medical School of Fudan University, Shanghai, China

Recent studies indicate that epicardium plays a crucial role in tissue regeneration after myocardial infarction (MI). After MI, epicardial cells can proliferate and undergo epithelial-to-mesenchymal transition to form epicardium-derived cells (EPDCs). These cells have potentials to differentiate into various types of cardiac cells. Obviously, amplification of endogenous regeneration through activating epicardium would be a novel strategy for the treatment of MI. In the present study, mesenchymal stem cells (MSCs) derived from mice were transfected with thymosin beta 4 gene (*Tβ4*) , Tβ4 was deemed to play an important role in activating epicardial cells after MI. Release of Tβ4 from the cells was determined with reverse phase high-performance chromatography (HPLC). Then, the transfected cells were seeded on PLACL/collagen nanofibers to prepare nanofiber-*Tβ4* transfected cell patches. Compatibility of the cells and nanofiber was evaluated with a scanning electron microscope and transmission electron microscope. Survival and proliferation of the cells on PLACL/collagen nanofibers was decided with MTT assay and a hypoxia model. The MI models of $Wt1^{CreERT2/+}$;$Rosa^{26mTmG/+}$ female mice were established by ligating left anterior descending coronary artery at 1 week after Tamoxifen administration. One week after MI, the nanofiber-cell sheets were transplanted on the epicardium of infarcted region of the transgenic mouse hearts. An indelible GFP label was used to trace activated epicardial cells and the fate of the EPDCs. Y chromosome fluorescence in situ hybridization was used to trace survival and differentiation of the transplanted cells in the infarcted tissues. The differentiation of the EPDCs and transplanted cells towards cardiomyocytes or vascular cells was evaluated with immunohistochemical staining. The concentration of Tβ4 in the transfected cells culture medium increased significantly. The cells on PLACL/collagen nanofibers spread well, and compatibility of the cells and nanofiber was well. The number of survival and proliferative cells on PLACL/collagen nanofibers was increased with culture time. At four weeks after implantation, expansion of the infarcted ventricle wall was prevented effectively in the nanofiber-cell pacth groups. Cardiac function in the nanofiber-*Tβ4* transfected cell pacth group was improved significantly, and scar area reduced obviously. In the nanofiber-*Tβ4* transfected cell pacth group, GFP^+ EPDCs cells in subepicardium increased,

and some of the GFP^+ cells expressed CD31 or α-SMA, some of the GFP^+ cells expressed cTnT. Fluorescence in situ hybridization showed that some Y chromosome-positive cells migrated into the epicardial and subepicardial region, and a few of them expressed CD31, α-SMA or cTnT, and Y chromosome-positive cells expressing CD31 incorporated into the microvessels in the infracted tissue. These results suggested that Tβ4 released slowly from PLACL/collagen nanofibers could effectively attenuated left ventricular remodeling and improved cardiac function by activating epicardial cells. The nanofiber-*Tβ4* transfected cell patches served as a mechanical barrier against progressive left ventricular dilation in infracted area, and induced effectively angiogenesis and regenerating cardiac tissue in infracted region. Our finding also provided a novel strategy for myocardial regeneration by enhancing the endogenous regenerative capacity of the epicardial lineage.

F-2288

A DECELLULARIZED MATRIX AS TISSUE ENGINEERING APPROACH FOR REMODELING DISEASED DIAPHRAGM OF MUSCLE-SPECIFIC SPINAL MUSCULAR ATROPHY MOUSE MODEL

Martina Piccoli[1], Luca Urbani[1], Mario E. Alvarez Fallas[1], Arben Dedja[2], Enrico Magrofuoco[3], Nicola Elvassore[3], Chiara Franzin[1], Paolo De Coppi[4], Michela Pozzobon[1]

[1]Fondazione Istituto di Ricerca Pediatrica Città della Speranza, Padova, Italy, [2]University of Padova, Dipartimento di Scienze Cardiologiche Toraciche e Vascolari, Padova, Italy, [3]University of Padova, Dipartimento di Ingegneria Industriale, Padova, Italy, [4]UCL Institute of Child Health and Great Ormond Street Hospital, London, United Kingdom

Impairment of extracellular matrix (ECM) is a major pathological feature in skeletal muscle degenerative disorders such as muscular dystrophy and other myopathies, frequently caused by mutations in genes encoding for ECM proteins. As a consequence, muscle ECM gets progressively substituted by fibrotic tissue, and therefore cellular or gene therapies are not effective. In particular, myopathy is especially critical for respiratory (e.g. diaphragm) and limb muscles as they become fibrotic and weak. Spinal muscular atrophy is an autosomal recessive disorder caused by mutations in SMN gene; it affects the spinal cord neurons and is clinically characterized by muscle weakness. In HSA-Cre, SmnF7/F7 animal model, only skeletal muscle is specifically affected by the disease and we have already seen that the muscles, in particular the diaphragm, display fibrosis and myofiber loss. Since acellular ECM scaffolds retain important bioactive constituents and factors, using a decellularized matrix obtained from healthy-mice diaphragm we aimed at ameliorating diaphragm muscle condition of HSA-Cre, SmnF7/F7 mouse model. We characterized the decellularized diaphragm muscle after detergent enzymatic treatment (DET) establishing that 3 DET cycles are a good compromise between DNA content reduction ($p<0.001$ vs fresh tissue) and ECM preservation. After 3 DET cycles, collagen and elastin content was not statistically different from fresh tissue, while GAGs showed a $p<0.05$ in respect to fresh tissue. Importantly, decellularized matrix possessed the same thickness and stiffness of a fresh diaphragm. In vivo: acellular patches were surgically applied to the affected diaphragm of HSA-Cre, SmnF7/F7 mice and changes in terms of thickness, morphological and cytological aspects of the native diaphragm after 7, 15 and 30 days were evaluated. New collagen deposition was noticeable 15 days post implantation with evident features that a remodeling process began. The acellular patch was gradually re-populated during the three time points and, after 30 days, was partially reabsorbed. On the other hand, the weak native diaphragm underwent remodeling and increased in thickness.
In conclusion, diaphragms were successfully decellularized and we can affirm that the ECM exerted a positive effect when applied in a myopathic diaphragm influencing cellular turnover and matrix composition.

F-2291

TRANSPLANTABLE DECELLULARIZED WHOLE-ORGAN SCAFFOLD FOR LIVER REGENERATIVE THERAPY IN LARGE ANIMAL MODEL

Hiroshi Yagi[1], Alejandro Soto-Gutierrez[2], Taizo Hibi[1], Yuta Abe[1], Katsunori Tanaka[1], Minoru Kitago[1], Masahiro Shinoda[1], Hideaki Obara[1], Osamu Itano[1], Minoru Tanabe[1], Yuko Kitagawa[1]

[1]Surgery, School of Medicine, Keio University, Shinjuku-ku, Tokyo, Japan, [2]Pathology, Center for Innovative Regenerative Therapies, Department of Surgery, Transplantation Section of Children's Hospital of Pittsburgh, McGowan Institute for Regenerative Medicine and the Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA, United States

Background and aims: New methods to facilitate recovery from end-stage organ failure are highly desirable, since only limited treatment options are available in this context. Recent progress in the field of tissue engineering has opened attractive approaches for clinical applications of regenerative medicine which could be an option in this regard. Of these, tissue decellularization technology, which retains all the necessary cues for cell maintenance and homeostasis, such as the three dimensional structure of the organ and its extracellular matrix (ECM) components, has recently been applied to whole organs. However, the use of this technology in the context of the digestive organs is still under investigation, being more difficult because of their complicated 3D structure and complex functioning. And there is an urgent need to find its feasibility of human scale-up and biological alterations of the scaffold after implantation.
Methods: In this study, we demonstrate that this decellularization technology could be applied in a large animal model to develop a transplantable engineered liver graft. Decellularized liver scaffold was generated by the same procedure as we had applied in rats using trypsin and tritonX-100, which was connected to portal vein for inflow and inferior vena cava for outflow with artificial vessel grafts. Anti-coagulants and antibiotics were applied prior to and during the surgery to prevent coagulation in the native matrix scaffold and infection after transplantation. Also we applied MPC polymer via portal vein of the liver scaffold to prevent coagulation after the surgical anastomosis. Histological study was performed at different time points through day 7 to evaluate cell infiltration and adhesion around the scaffold as well as blood inflow and out flow.
Results: The decellularization technology could be scaled up in size with preserved basement membrane and 3D structure demonstrating by SEM and TEM. More importantly, the liver graft was successfully transplanted in porcine body by vessel anastomosis without bleeding through day 7. Although histological study revealed massive adhesion with infiltration of inflammatory cells including lymphocytes and fibroblasts especially in the edge of the scaffold and the coagulations were not totally avoided in the transplanted graft, the graft was well perfused and preserved in the porcine abdominal cavity without bleeding or absorption.
Conclusions: Although it requires improvement and customization with regard to anti-coagulation and further applicable cell sources, we could scale-up and optimize the system to apply this unique technology for clinical applications. Recent progress in stem cell technology will facilitate this new method as a novel platform of functional organ graft for organ failure.

F-2292

MODELING DESMOSOME DISEASE IN VITRO WITH INDUCED PLURIPOTENT STEM CELL TECHNOLOGY AND TISSUE ENGINEERING

Nathaniel Huebsch[1], Yuchiro Miyaoka[1], Matthew J. Spindler[1], Zhen Ma[2], Mohammed A. Mandegar[1], Luke Judge[1], Caitlin R. Russell[1], Paweena Lizarraga[1], Trieu Nguyen[1], Jennie Yoo[1], Po-Lin So[1], Kevin E. Healy[2], Bruce R. Conklin[1]
[1]Gladstone Institute, San Francisco, CA, United States, [2]Bioengineering, University of California, Berkeley, Berkeley, CA, United States

Cardiomyopathies are a complex set of diseases in which mechanical stresses on the myocardium, genetics and other factors interact in a combinatorial manner to cause functional and structural abnormalities that cause heart failure. Induced pluripotent stem (iPS) cell and genome engineering technologies have been applied to model a variety of cardiomyocyte disease phenotypes, including cardiomyopathy, in vitro. Although these studies have focused on genetic causes of the disease there are additional biophysical constraints that likely also contribute to disease phenotypes. We hypothesize that mechanical constraints - specifically, tissue geometry and cardiomyocyte alignment - act in concert with desmosome proteins to maintain normal cell-cell contacts, gap junctions and cardiomyocyte gene expression.

We are investigating this hypothesis with a combination of tissue and genome engineering techniques. On the genetic level, we are exploring the effects of mutations in the desmosome component Plakophilin 2 (PKP2), a gene associated with arrhythmogenic right ventricular dysplasia (ARVD). To control the geometry of cardiomyocyte micro-tissues, we will use existing microfabrication strategies to pattern extracellular matrix (ECM) proteins (e.g. fibronectin) “islands” of different aspect ratios to force differential levels of alignment in iPS-CM.
To achieve our goals we required >10^7 human iPS-CMs. Thus, we first optimized existing methods for efficient iPS differentiation toward the cardiomyocyte lineage, density gradient iPS-CM enrichment and cryopreservation. Large-scale iPS-CM differentiation using a small-molecule based protocol (Lian et.al. Proc. Natl. Acad. Sci. USA 2012) to yield populations of at least 3 x 10^7 cells with a fraction of 60-80% expressing cardiac troponin (cTnT) 15 days after initiating differentiation with the GSK-3β inhibitor Percoll fractionation (Zhu et al. Methods. Mol. Biol. 2011) was then applied to further enrich for cardiomyocyteswith populations having initial purity > 50% yielding a final population where more than 90% of cells expressed cTnT.
To assess the function of micro-tissues non-invasively, we have adapted and improved motion tracking algorithms based on block-matching (Okanu et al. Tissue Eng. C.2012). Procedures were validated using drugs with known cardiotoxic or stimulatory function. Using these algorithms, we confirmed data from previous work indicating that iPS-CM recovery from cryopreservation in 10% DMSO was enhanced by inhibiting ROCK signaling with Y27632 (Kim et al. Reprod. Sci. 2011), as measured by cell viability and recovery of maximum contraction rate. .
In vivo, cardiomyocytes achieve a specific morphology, and using micro-patterning strategies to force cells to achieve this morphology has been shown to maximize cardiomyocyte traction forces in vitro (Kuo et al. Am J. Pathol. 2012). By forcing iPS-CM to achieve maximal traction forces and alignment with one another in micro-tissues, we expect to maximize stresses transmitted via desmosomes, thus making iPS-CM that are genetically predisposed to ARVD (e.g. PKP2 mutant cells) more likely to display functional hallmarks of this disease in vitro.

F-2293

ENGINEERING TISSUE STRUCTURE FORMATION OF THE PROGENITOR SALIVARY GLAND CELLS BY DEACETYLATED CHITIN BIOMATERIALS

Tsung-Lin Yang[1], You-Tzung Chen[2], Ya-Chuan Hsiao[3]

[1]Department of Otolaryngology, National Taiwan University, Taipei, Taiwan, [2]Graduate Institute of Medical Genomics and Proteomics, National Taiwan University, Taipei, Taiwan, [3]Department of Ophthalmology, Zhongxing Branch, Taipei City Hospital, Taipei, Taiwan

ABSTRACT

Salivary gland dysfunction is a challenging clinical problem featured by an irreversible damage of salivary glands. No effective treatments have been established currently for dysfunctional salivary glands. Salivary gland is an important glandular organ responsible for regulating saliva to maintain physiological homeostasis. Branching structure is the characteristic tissue feature among many different glandular organs, and by which different types of cells can be organized to efficiently fulfill physiological functions. To regenerate functional salivary glands, recapitulation of tissue-specific structure formation is required. This study aims to engineer tissue structure formation of progenitor salivary cells by deacetylated chitin biomaterials. Chitosan is a deacetylated derivative of chitin with wide biomedical applications. Our previous studies had demonstrated its potential in regenerating salivary glandular structures. Because of its deacetylated nature, chitosan has unique properties when prepared with differing degrees of deacetylation (DDA). Although many biological responses that are mediated by chitosan can be affected by DDA, the impact of DDA on the structure formation of salivary glands remained unclear. In this study, the progenitor cells of salivary glands from murine submandibular gland (SMG) were used to investigate the role of chitosan DDA in regulating tissue structure formation. When chitin substrates with different DDA were used, the branching numbers of cultured SMG explants changed. Similar effects were observed in the culture with chitosan prepared from different degrees of acetylation. The mRNA and protein expression of type I and type III collagen were elevated in SMG explants with enhanced branching morphogenesis. In addition to the amounts of collagen, type I and type III collagen fibers were spatially present in the epithelial-mesenchymal junction of developing branches cultured with chitosan

of a specific range of DDA. The branch-promoting effect of chitosan DDA was abolished when SMG explants were treated with collagenase, both early in the stage of branch initiation and that of tissue structure establishment. This study demonstrates the impact of chitosan DDA on tissue structure formation of engineered salivary gland cells. The different proportions of side-chain components of chitin derivatives regulated structural formation of cultured SMG, indicating that DDA is an important parameter of chitin biomaterials to engineer tissue morphogenesis.

Embryonic Stem Cell Pluripotency

F-2295

ENHANCER TRANSCRIBED RNAS IN EMBRYONIC STEM CELLS ARE REGULATED BY THE TET FAMILY OF PROTEINS

Sridhar Rao[1], Kirthi Pulakanti[2], Luca Pinello[4], Cary Stelloh[3], Steven Blinka[5], Jeremy Allred[5], Samuel Milanovich[6], Sid Kiblawi[3], Jonathan Peterson[3], Guo-Chen Yuan[7]
[1]BloodCenter of Wisconsin, Milwaukee, WI, United States, [B]BloodResearch Institute, [C]Blood Research Institute, [1]BloodCenter of Wisconsin, Milwaukee, WI, United States, [2]Dana Farber Cancer Institute, Boston, MD, United States, [3]Medical College of Wisconsin, Milwaukee, WI, United States, [B]Department of Pediatrics, [3]Medical College of Wisconsin, Milwaukee, WI, United States, [4]Dana Farber Cancer Institute, Boston, MA, United States

Enhancers are cis-acting DNA elements that regulate transcription over vast distances, with recent work demonstrating they can be transcribed by RNA Polymerase II (Pol II), producing a non-coding RNA termed enhancer-transcribed RNAs (eRNAs). The objective of this study was to gain insight into whether eRNA producing enhancers are distinct from other types of cis-regulatory elements we sought to comprehensively identify and characterize them. We utilized ChIP-Seq datasets of different epigenetic marks and transcription factors to identify enhancers within embryonic stem cells (ESCs). Next, we identified lineage-specific eRNAs in ESCs and the somatic cell mouse embryonic fibroblasts (MEFs) using an existing RNA-seq dataset. These results illustrate that eRNA production occurs at a subset of enhancers identified by a variety of epigenetic marks (H3K4me1, H3K27Ac) or protein binding sites (p300, Nanog-Oct4-Sox2), but also at insulator elements occupied by CTCF. Among enhancers bound by Pol II is a subset that produce an eRNA exclusively in ESCs, implying they are lineage restricted. Genes linked to these enhancers are strongly down regulated in ESCs after exposure to retinoic acid to a greater degree than genes whose enhancer produced no eRNAs. However, we found that enhancers that produced an eRNA exclusively in ESCs showed low overall DNA methylation; in contrast enhancers that did not produce eRNAs were fully DNA methylated. We also found that the eRNA producing enhancers were typically occupied by the DNA hydroxylase Tet1, although their overall levels of 5'-hydroxymethylcytosine was equivalent to other enhancers. By focusing on an eRNA producing enhancer linked to the Nanog locus, we were able to determine that both Tet1 and Tet2 occupied this enhancer in ESCs, and depletion of Tet1 or Tet2 reduced eRNA production from this region. Collectively, this work implies that eRNA producing enhancers are distinct from other enhancer regions and exhibit lower levels of DNA methylation. This may represent distinct epigenetic mechanisms used to establish and/or de-recruit these enhancers during development. Experiments are underway to determine if eRNAs are functionally important in ESCs.

F-2296

MOLECULAR MECHANISMS UNDERLYING STEM CELL PLURIPOTENCY

Eric Bradley, Erhard Bieberich, **Guanghu Wang**
Georgia Regents University, Augusta, GA, United States

Understanding the molecular basis of stem cell pluripotency is fundamental to stem cell biology and early embryonic development, as well as to the application of regenerative medicine. We report here that the molecular chaperone heat shock protein 90 (Hsp90) is required for embryonic stem cell (ESC) pluripotency through regulating multiple pluripotency factors, including Oct4, Nanog and phospho-Stat3. Inhibition of Hsp90 by either 17-*N*-Allylamino-17-demethoxygeldanamycin (17-AAG) or miRNA led to ESC differentiation. Over-expression of Hsp90β

partially rescued the phenotype; in particular, the levels of Oct4 and Nanog were restored. Notably, Hsp90 associated with Oct4 and Nanog and protected them from degradation by the proteasome pathway, demonstrating that Oct4 and Nanog are potential novel Hsp90 client proteins. In addition, Hsp90 inhibition reduced the mRNA level of Oct4 but not Nanog. Hsp90 inhibition also increased expression of mesodermal lineage markers, implying that Hsp90 suppresses mesodermal differentiation from ESCs. These findings support a new role for Hsp90 in maintaining ESC pluripotency by sustaining the level of multiple pluripotency factors, especially Oct4 and Nanog. Supported by an AHA grant and an intramural grant to GW.

F-2297

IS HEY2 A NOVEL PLURIPOTENCY FACTOR IN HUMAN EMBRYONIC STEM CELLS?

Fiona M. Docherty, Ludovic Vallier
Surgery, University of Cambridge, Cambridge, United Kingdom

Transcriptional repressor protein, HEY2, is a known target of the Notch signalling pathway and is involved in cardiac development and disease. In our efforts to use human embryonic stem cells (hESC) to model cardiac development we have uncovered a novel role for HEY2 in these pluripotent cells.
Our initial investigations by RT-qPCR analyses and immuno-fluorescent staining led to the finding that HEY2 is expressed in pluripotent cells. To determine if HEY2 is playing an active role in hESC, we performed loss and gain of function experiments. We found that knock down of HEY2 expression using shRNA induced spontaneous differentiation of hESC while HEY2 overexpression served to enhance their pluripotent phenotype. We also demonstrated that HEY2 improves the efficiency of fibroblast reprogramming to iPSC when used in combination with the four conventional reprogramming factors: OCT4, KLF4, SOX2 and cMYC. Further studies into the transcriptional regulation of HEY2 showed that its expression is increased by FGF and decreased by inhibition of the Activin/Nodal signalling pathway. We also observed evidence of synergistic interactions between FGF and HEY2 in maintaining NANOG expression upon inhibition of Nodal signalling.
Taken together, these results suggest a role for HEY2 in maintaining pluripotency of hESC. This work has important implications in improving the efficiency of human iPSC derivation and our understanding of the fundamental pathways associated with hESC pluripotency. Our future work will focus on determining the signalling pathways by which HEY2 can act on pluripotency and differentiation.

F-2298

MAINTENANCE OF STEM CELL PLURIPOTENCY IN STIRRED SUSPENSION BIOREACTORS VIA MECHANOTRANSDUCTION

Bradley Day, Derrick E. Rancourt
University of Calgary, Calgary, AB, Canada

Pluripotent stem cells (PSCs) including embryonic stem cells and induced pluripotent stem cells are capable of self-renewal and differentiation into any cell type. The adherent culture system currently used to generate PSCs does not efficiently produce an adequate number of cells and lacks culture control leading to culture heterogeneity. To overcome this application roadblock, we have developed stirred suspension bioreactors (SSBs) as a method for culturing PSCs as aggregates. Cells are stirred at a shear stress level of 6 dyne/cm2 to maintain an optimal aggregate size for mass transfer of oxygen and nutrients. Using murine PSCs, we have observed that pluripotency is maintained in the absence of leukemia inhibitory factor, an obligate pluripotency maintenance factor. We have been able to replicate this in 100 mL and 10 mL SSBs. Our results suggest that shear stress may play a role in the maintenance of pluripotency. We hypothesize that shear stress alters cell signalling and gene expression via mechanotransduction, which alters adherens junctions and allows β-catenin to translocate to the nucleus and regulate pluripotency. We have examined the nuclear translocation of β-catenin in murine ESCs using a TCF/LEF GFP reporter system and have observed a 40-fold increase in the amount of nuclear β-catenin in cells exposed to higher shear stress levels. Confocal microscopy reveals that β-catenin activation occurs throughout the aggregate, suggesting

that the affect occurs uniformly. This observation is consistent with a recent report using other cells indicating that E-cadherin may have mechano-sensing properties, which lead to a restructuring of adherens junctions and concomitant nuclear translocation of β-catenin.

F-2301

TRANSCRIPTOME DIVERSITY AT MULTI AND SINGLE CELL LEVELS IN RABBIT PLURIPOTENT STEM CELLS

Yann TAPPONNIER[1], Pierre OSTEIL[1], Barbara SCHMALTZ-PANNEAU[2], Luc JOUNEAU[2], Cédric CABAU[3], Cécile DONNADIEU[4], Hervé ACLOQUE[5], Véronique DURANTHON[2], Marielle AFANASSIEFF[1], Pierre SAVATIER[1]
[1]Stem cell and Brain Resarch Institute, INSERM U846, INRA USC1361, BRON, France, [2]Biology of Development and Reproduction, INRA, JOUY-EN-JOSAS, France, [3]SIGENAE, INRA, NOUZILLY, France, [4]Plateforme Génomique, Génopole Toulouse/Midi Pyrénnées, TOULOUSE, France, [5]UMR444, INRA, CASTANET TOLOSAN, France

Pluripotent stem cells (PSCs) can exist in at least two morphologically, molecularly and functionally distinct pluripotent states, designated as the naïve and primed states; PSC exist in both states in rodents, whereas only the primed stated has been described in primates so far. Not much is known about the pluripotent state of PSCs in rabbits. To address this, we derived and characterized 2 types of rabbit PSCs from the same breed of New Zealand White rabbits: 3 lines of induced PSCs (rbiPSCs) that were obtained by reprogramming adult skin fibroblasts, and 4 lines of embryonic stem cells (rbESCs). All cell lines required FGF2 for their growth and proliferation. We purified the SSEA subpopulations by FACS sorting in each line, and performed a global analysis of their expression profiles. For this, we used a rabbit-specific gene expression microarray containing approximately 13,000 independent genes. Hierarchical clustering showed that all subpopulations of rbESC lines were clustered on one side and all subpopulations of rbiPSC lines were clustered together on the other. This indicates that the variability is mainly associated with the cell status (rbESC vs rbiPSC) and not with the SSEA expression status. We next performed a qPCR analysis of Blimp1, Cdx2, Cdh1 (E-cadherin), Cdh2 (N-cadherin), Cldn6, Dax1 (NrOb1), Dazl, Essrb, Fbxo15, Fgf4, Gbx2, Klf4, Lefty2, Nanog, Oct4 (Pou5f1), Otx2, Pecam1, Pitx2, Piwil2, Rex1 (Zfp42), Tbx3, and Tcfcp2l1, which have been used to evaluate stemness and demarcate the naïve and primed pluripotent states in rodents. In agreement with the microarray data, correlation clustering of the Delta Ct values showed that the rbiPSC subpopulations exhibited fewer differences between them than with the rbESC subpopulations. Moreover, when compared to the rabbit inner cell mass (ICM), the rbiPSCs were the closest; all rbESC subpopulations, irrespective of their SSEA1 status, were more distantly related to the ICM than the SSEA1+ rbiPSCs. Of note, both rbiPSCs and ICM cells expressed the naive markers Cdh1, Essrb, Cldn6, Klf4, Dazl, Piwil2, and Pecam1. However, rbiPSCs did not express all the molecular markers of naïve pluripotency, including Rex1, Tbx3, Gbx2, Fgf4, and Dax1. Based on these observations, we propose that rabbit iPSCs self-renew in an intermediate state between naïve and primed pluripotency. To gain further insight into cell diversity within the rabbit PSC populations, we started examining the expression of pluripotency markers by single-cell qRT-PCR. Preliminary results showed that rabbit ESC populations contained some rare cells, which co-expressed the naive markers Essrb and Tbx3. Our ongoing experiments aim to identify these cells both in iPS cell populations and in the preimplantation embryo. This work should help decipher the molecular signature of naïve pluripotency in rabbits and, ultimately, will lead to generate PSC lines suitable for blastocyst colonization and chimaera production.

F-2302

THE H19 IMPRINTED GENE AFFECTS THE PLURIPOTENCY STATE OF HUMAN EMBRYONIC STEM CELLS (HESCS) AND HUMAN EMBRYONAL CARCINOMA CELLS (HECCS) IN VITRO AND THE GROWTH OF HESC-INDUCED TERATOMAS AND HECC-INDUCED TERATOCARCINOMAS IN VIVO

Michal Gropp[1], Evelyne Zeira[2], Benjamin Reubinoff[1], Eithan Galun[2]
[A]The Sidney and Judy Swartz Human Embryonic Stem Cell Research Center, [B]The Goldyne Savad Institute of Gene Therapy, [1]Hadassah-Hebrew University Hospital, Jerusalem, Israel

The H19 imprinted gene encodes a long non-coding RNA, which is highly expressed in placenta and embryonic tissues and is down-regulated after birth. However, H19 was found to be re-expressed in several cancers. Conflicting data suggest that H19 functions as a tumor suppressor as well as an oncogene. Yet, at present, the physiological role of H19 is unknown.
We aimed to investigate the role of H19 in normal early embryogenesis as well as in tumorogenesis using two experimental systems: human embryonic stem cells (hESC), normal pluripotent cells derived from pre-implantation embryos, and their malignant counterparts, human embryonal carcinoma cells (hECCs), pluripotent cells derived from teratocarcinomas. Both cell types can self-renew for long periods yet can differentiate into cells representing the three germ layers. *In vivo*, mice transplanted with hESCs develop benign teratomas containing disorganized differentiated cells and tissues, while mice transplanted with hECCs develop teratocarcinomas which contain differentiated cells and foci of malignant hECCs. Thus, hESC-induced teratomas and hECC-induced teratocarcinomas may serve as models to study both normal early human embryogenesis and tumorogenesis.
A tetracycline (tet) inducible lentiviral RNAi system was used to target H19 in hESCs and hECCs. Transduction of HES-1-hESC line and NCCIT-hECC line by lentiviral vectors, harboring a tet-inducible H19-shRNA or a control luciferase-shRNA, and a constitutive tet-repressor fused to GFP (HLTV-shH19 and HLTV-shLUC) was highly efficient, resulting in over 90% of cells expressing GFP during long culturing periods. Induction of H19-shRNA expression by Doxycycline (Dox) down-regulated H19 expression in HES-1/HLTV-shH19 by 56% and in NCCIT/HLTV-shH19 by 48%, compared to control cells. Real-time PCR analyses revealed that the decrease in H19 expression in both cell lines was followed by a decrease in the expression levels of the key pluripotency transcription factors Oct4 and Nanog. FACS analysis further showed a decrease in the percentage of cells expressing the pluripotency-associated cell-surface markers Tra-1-60 and Tra-1-81. Thus, it seems that down-regulation of H19 in undifferentiated hESCs and hECCs affects their pluripotency state *in vitro*.We next transplanted immunodeficient SCID-Beige mice subcutaneously with HES-1 or NCCIT cells tranduced with HLTV-shH19 or control vector, and induced shRNA expression by dox. Tumors development was monitored for 7-11 weeks, and mice were sacrificed when tumors reached 2.5 cm^3. The results of two transplantation experiments revealed that teratomas generated from HES-1/HLTV-shH19 developed faster than teratomas generated from control cells, as reflected by a significantly higher fraction of the animals sacrificed at earlier time points. In contrast, the kinetics of teratocarcinoma formation in mice transplanted with NCCIT/HLTV-shH19 was slower compared to control cells, with significant increase in the fraction of surviving animals. We are currently performing histological analysis of the tumors to characterize the effect of H19 down-regulation on differentiation. These results suggest opposing roles for H19 *in vivo*: it inhibits the growth of hESC-derived teratoma while promoting the growth of hECC-teratocarcinoma.

F-2303

SIRT1 AND SIRT2 DUAL INHIBITION INDUCE PLURIPOTENT STEM CELL APOPTOSIS VIA P53 ACETYLATION

Yong Joon Huh, Hyun Sub Hwang, Dong-Wook Kim
Department of Physiology, Brain Korea 21 Project for Medical Science, Yonsei University, Seoul, Korea, Republic of

Sirtuin (Sirt1/2) NAD+ dependent deacetylase plays an important role in cell survival under stress conditions. While Sirt2 is ubiquitously expressed, Sirt1 recently has been found to be over-expressed in embryonic stem cells(ESCs) and thus these Sirtuins may play key roles in survival and maintenance of pluripotent stem cells (PSCs, embryonic stem cells and induced pluripotent stem cells). In this study, we investigated the role of Sirt1/2 in PSC survival by chemical inhibitions. We found that only dual inhibitors of Sirt1 and 2 (Sirt1/2): Sirtuin, Salamide, and Teniovin6 affected the viability of PSCs. While Sirt1/2 inhibition led to apoptosis of undifferentiated PSCs, it did not affect differentiated cells. To prove Sirt1/2 inhibition-mediated apoptosis in PSCs, we tested whether p53-related pathway is regulated by Sirt1/2 deacetylation. Sirt1/2 down regulation led to reactivation of p53 followed by overexpression of p53 target genes: PUMA and BAX. In the end, cleaved caspase-3 was clearly detected in PSCs treated with Sirt1/2 inhibitors. Pro-apoptotic gene (PUMA, BAX) over-expressions and caspase3 activation imply that the apoptosis is p53-mediated event in PSCs. Thus, unlike undifferentiated cells, PSCs are susceptible to Sirt1/2 inhibition-mediated p53 activation that leads to apoptosis of PSCs. To validate the PSC-specific apoptosis induction by Sirt1/2 inhibitors, we utilized embryoid bodies containing partial differentiated cells and undifferentiated cells. PSC survival was ex-

amined by PSC marker (Oct4, SSEA4) expressions. In day 6 EBs, Sirt1/2 inhibitors dramatically reduced Oct4 levels. Activated caspase-3 was mostly detected in SSEA-4+ cells among the mixed cell population implying the specificity of Sirt1/2 action to PSCs. Our findings provide a novel strategy to eliminate undifferentiated cells by Sirt1/2 inhibition in the process of *in vitro* differentiation.
This research was supported by grants from the Bio & Medical Technology Development Program of the National Research Foundation (NRF), Ministry of Education, Science and Technology (2012M3A9B4028631, 2012M3A9C7050126) and Korea Health Technology R&D Project, Ministry of Health and Welfare, Republic of Korea (A120254).

F-2305

ROLE OF REPTIN/PONTIN IN THE REGULATION OF SELF-RENEWAL AND PLURIPOTENCY OF EMBRYONIC STEM CELLS

Eun Kyoung DO, hyo Cheon Cheon, Jae Ho Kim
Medical Research Center for Ischemic Tissue Regeneration, BK21(Brain Ko, School of Medicine, Pusan National University, Yangsan, Korea, Republic of

Oct4 has been implicated in the regulation of pluripotency in embryonic stem (ES) cells and reprogramming of somatic cells into induced pluripotent stem cells. However, the molecular mechanisms involved in the Oct4-dependent regulation of pluripotency and reprogramming have not been clear. To gain insight into mechanisms regulating Oct4 protein activity in mouse ES cells, in the present study, we tried to identify Oct4-binding proteins using immunoprecipitation and proteomic analysis. Tandem affinity tagged exogenous Oct4 gene was overexpressed in HEK293 cells, and Oct4-binding protein complexes were purified from Oct4-transfected 293FT cells using immunoprecipitation. Molecular identities of the Oct4-binding proteins were elucidated by mass spectrometry. We identified Reptin and Pontin, key components of ATP-dependent chromatin remodeling complexes, as the Oct4-binding proteins. Depletion of endogenous Reptin/Pontin using lentiviral small hairpin RNA or small interfering RNA inhibited the alkaline phosphatase activity in ES cells. Furthermore, we investigated that shRNA and siRNA mediated silencing of Reptin/Pontin decreased the expression of pluripotency-specific marker genes, including Oct4, Sox2, and Nanog. Depletion of Reptin/Pontin suppressed not only pluripotency in ES cells but also somatic cell reprogramming of fibroblasts to induced pluripotent stem cells. These results suggest that Reptin/Pontin play a key role for maintenance of pluripotency of ES cells and reprogramming of somatic cells by regulating Oct4-dependent gene transcription.

F-2306

A SELF-SUSTAINING FEEDBACK LOOP THAT REGULATES PROTEOME DIVERSITY AND SUPPORTS SELF-RENEWAL IN PLURIPOTENT STEM CELLS

Yu Lu[1], Chance John Luckey[1], James J. Collins[2], George Q. Daley[3], Jarrod A. Marto[4]
[1]Pathology, Brigham Women's Hospital, Boston, MA, United States, [2]Biological Engineering, Boston University, Boston, MA, United States, [3]Hematology/Oncology, Children's Hospital, Boston, MA, United States, [4]Cancer Biology, Dana-Farber Cancer Institute, Boston, MA, United States

Ample biochemical data has established that OCT4, NANOG, and SOX2, the master regulators of pluripotency, interact with a large number of proteins, form transcription regulatory loops, and link to multiple pathways. Nonetheless discrepancies between ostensibly identical pluripotent cell lines, in addition to the divergent lineage commitment properties of iPSC derived from different adult tissues, illustrate that the molecular network supporting self-renewal is not yet fully resolved.
Herein we define a molecular signature for pluripotent cells based on gene expression, protein expression, and quantitative phosphorylation profiles. Interpretation of these data in the context of macromolecular networks in-

dicates that signature genes within the RNA splicing pathway are enriched for physical interactions with known pluripotent factors.
SFRS2 is the top representative of splicing factors among pluripotency signature genes. OCT4 drives SFRS2 expression, and RNAi against SFRS2 compromises pluripotency. Analysis of exon junction microarray data revealed that nearly 3000 genes are subject to alternative splicing in human ESC and further that these are also enriched for physical interactions with known pluripotent factors. Amongst this subset of pluripotency signature genes, the methyl-CpG binding domain protein 2 (MBD2) exhibited the strongest cell-type specific alternative splicing in an SFRS2-dependent manner. The two distinct isoforms of MBD2 induced different phenotypes in human ESC and during the reprogramming of somatic cells. CHIP and coimmunoprecipitation analyses indicated that these isoforms share similar DNA binding yet divergent interactions with the transcription repressive Nucleosome Remodeling and Deacetylation (NuRD) complex. Moreover, we observed that miR-302 cluster members selectively targeted the MBD2a isoform expressed in somatic cells, while conversely members of miR-301 cluster reduced SFRS2 expression in human ESC in the context of OCT4 depletion.
Collectively our data support a model in which pluripotency master regulators participate in a self-sustaining feedback loop, regulating splicing factors (e.g., SFRS2) and the microRNA machinery to mediate protein diversity via alternative splicing (e.g., MBD2) to enforce a pluripotent ground state.

F-2307

GBX2, A LIF/STAT3 TARGET, PROMOTES REPROGRAMMING TO AND RETENTION OF THE PLURIPOTENT GROUND STATE.

Chihi Tai, Qilong Ying
Department of Cell & Neurobiology, Keck School of Medicine, University of Southern California, Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research at USC, Los Angeles, CA, United States

Activation of signal transducer and activator of transcription 3 (Stat3) by leukemia inhibitory factor (LIF) maintains mouse embryonic stem cell (mESC) self-renewal and also facilitates reprogramming to ground state pluripotency. Exactly how LIF/Stat3 signaling exerts these effects, however, remains elusive. Here, we identified gastrulation brain homeobox 2 (Gbx2) as a LIF/Stat3 downstream target that when overexpressed allows long-term expansion of undifferentiated mESCs in the absence of LIF/Stat3 signaling. Elevated Gbx2 expression also enhanced reprogramming of mouse embryonic fibroblasts to induced pluripotent stem cells. Moreover, overexpression of Gbx2 was sufficient to reprogram epiblast stem cells (EpiSCs) to ground state ESCs. Our results reveal a novel function of Gbx2 in mESC reprograming and LIF/Stat3-mediated self-renewal.

F-2308

DISSECTING PHOSPHORYLATION MECHANISMS GOVERNING EMBRYONIC STEM CELL IDENTITY

Dung-Fang Lee, Jie Su, Julian Gingold, Yen-Sin Ang, Henia Darr, Christoph Schaniel, Ihor R. Lemischka
Mount Sinai School of Medicine, New York, NY, United States

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), characterized by their ability to self-renew indefinitely and differentiate into all cell lineages of an organism, hold great promise for biomedicine as an unlimited source of cells for generating differentiated progenies for transplantation-based therapies, studying the etiology of various diseases as well as for developing new drug treatments. It is essential to have a comprehensive understanding of the molecular mechanisms controlling stem cell pluripotency and differentiation into a wide range of mature cells in order to fulfill ESC/iPSC's promise in regenerative therapy and clinical application.
In our previous study (Cell Stem Cell. 2012 Aug 3;11(2):179-94), we developed a short hairpin (sh) RNA functional genomics screening strategy that integrates competition and genetic complementation systems to identify the essential cellular signaling pathways that are required for maintaining ESC self-renewal and pluripotency (Nat Protoc. 2012 Mar 22;7(4):729-48). Our screening led us to the discovery of a novel regulatory function of the kinase Aurka. We demonstrated that Aurka acts via the p53 pathway in ESCs and that a single phosphorylation event mediated by

Aurka on p53 shifts ESCs from differentiating- to a self-renewal state. Also, suppression of p53 function by Aurka-mediated phosphorylation is required for somatic cell reprogramming. When such regulation is compromised, the reprogramming process of forming iPSCs is impeded.
To understand the complexities of ESC self-renewal machinery, we continued to explore other 4 phosphoregulators (Chek1, Bub1b, Ppm1g and Ppp2r1b) which are identified in our ESC self-renewal screening. In order to elucidate the underlying mechanisms by which these 5 phosphoregulators control ESC self-renewal, we applied systematic approaches to integrate the changes in genome-wide gene expression profiles upon depletion of these 5 phosphoregulators using a variety of computational approaches. These analyses demonstrate the importance of maintaining genome stability in ESC self-renewal. Indeed, depletion of these 5 phosphoregulators led to the accumulation of DNA damage and genomic abnormalities. We further examined the effects on ESC self-renewal after depletion of numerous genome maintenance and stability-associated families, including molecules involved in DNA replication and checkpoints, mRNA processing, Fanconi anemia, and Charcot-Marie-Tooth disease associated gene-products. We show different degrees of compromised ESC self-renewal and pluripotency upon depletion of these molecules. These findings suggest that the maintenance of genome integrity is essential for ESC self-renewal and pluripotency. Furthermore, the decision by ESCs to transition from pluripotency to differentiation may be a novel mechanism to effectively remove compromised stem cells from a position of dramatic organismal damage potential.
In summary, our studies reinforce the need to develop safe and effective methods for utilizing ESCs for regenerative medicine. Additionally, understanding the basic molecular mechanisms underlying ESC regulation will provide invaluable information to bring induced pluripotent stem cells (iPSCs) towards future clinical applications.

F-2311

EFFICIENT GENERATION OF HUMAN EMBRYONIC STEM CELLS FROM SINGLE BLASTOMERES OF POOR-QUALITY CLEAVAGE EMBRYOS

Hossein Baharvand[1], Adeleh Taei[2], Seyedeh-Nafiseh Hassani[2], Poopak Eftekhari-Yazdi[2], Najmeh-Sadat Masoudi[2], Mohsen Nokhbatolfoghahaei[3], Hamid Gourabi[2], Mojtaba Rezazadeh Valojerdi[2]
[1]Royan Institute, Tehran, Iran, Islamic Republic of, [2]Royan institute, Tehran, Iran, Islamic Republic of, [3]Shiraz university, Shiraz, Iran, Islamic Republic of

Abstract
Human embryonic stem cells (hESCs) are mainly derived from the inner cell mass of surplus blastocysts which obtained from the infertility clinics. One fascinating approach to attain hESCs without injuring a living embryo is the use of single blastomeres (SBs) that removed from the embryos by a method similar to preimplantation genetic diagnosis technique, although the low efficiency of hESC generation by this method limits its feasibility. In this study, we sought to improve the conditions for increasing the survival and proliferation of SBs in respect of hESC derivation. To this end, two different strategies were devised. In the first strategy (chemical approach), we evaluated the effects of some small molecules, that have shown reinforcement of reprogramming during the generation of induced pluripotent stem cells, on hESC generation from SBs. Single blastomeres of poor-quality cleavage stage embryos (6-8 cells) were mechanically dispersed and individually grown in hESC medium contained small molecules. We revealed that GSK3 inhibitor, CHIR99021, in combination to Rock inhibitor, Y27632, could have impressive effect on proliferation and hESC line generation from SBs. However, although the generated lines showed all of the key stemness characteristics, they usually could not preserve the genomic integrity maybe because of the GSK3 inhibition. In the second strategy (physical approach), we considered O2 gas tension on SB culture condition. We identified that reduction of O2 gas tension could play powerful role on SB-derived hESC line generation. We observed 75% of cleavage embryos led to hESC line establishment from SBs. Furthermore, this approach stabilized genomic integrity in passaging cell lines as well as other pluripotency markers. Overall, the affirmative effect of O2 gas tension modification in the way similar to in vivo growth of early embryo suggested that hESC derivation from SB could be feasible if we could identify and mimic the condition of natural early development. Further recognition

of early embryo requirements could hold promise for hESC generation from biopsied SB of living embryos without injuring them.

F-2312

GENETIC ANALYSIS OF STAT3-DEPENDENT AND -INDEPENDENT MECHANISMS OF EMBRYONIC STEM CELL PLURIPOTENCY

Alissa M. Pham, Sang Yong Kim, Isabelle Marie, David E. Levy
Pathology, New York University Medical Center, New York, NY, United States

Maintaining pluripotency of murine embryonic stem cells (mESCs) in vitro can be achieved by artificially supplementing the culture milieu with factors that deflect differentiation-inducing signals. In conjunction with bone morphogenetic protein, activation of the leukemia inhibitory factor (LIF)/STAT3 signaling cascade drives optimal mESC self-renewal, and plays a critical role in establishing pluripotency during molecular reprogramming. In particular, STAT3 has been implicated as a transcriptional activator of numerous pluripotency genes, and has been documented to bind their promoters. However, despite these key functions in mESC survival, recent reports demonstrated STAT3 can be dispensable for mESC maintenance and propagation. Long-term mESC growth can be sustained in the absence of LIF, with the use of inhibitors against glycogen synthase kinase 3 (GSK3) and MAP/ERK kinase (MEK) signaling. Moreover, under these conditions, mutant mESCs lacking STAT3 remain undifferentiated. This evidence raised questions concerning the centrality of STAT3 in maintaining mESC pluripotency, and whether the mechanisms dictating this fate involve overlapping or independent signaling pathways. With the derivation of STAT3-null mESCs and subsequent gene expression and transcriptional profiling, we can address this question by comparing the transcriptional regulatory mechanisms that result from LIF/STAT3 signaling relative to inhibition of MEK and GSK3 kinase function. More specifically, we aim to identify the underlying molecular mechanisms by which these distinct environmental conditions lead to pluripotency, whether they share a common gene signature, and if they achieve the same outcome by convergent or distinct mechanisms. As the self-renewal of human ESCs (hESCs) and mESCs is contingent on different environmental factors, delineating the core requirements for mESC self-renewal may reveal conserved endogenous pluripotency networks, and provide key applications that will facilitate the use of hESCs in cell-based therapies and disease modeling.

F-2313

ROLE OF STRESS INDUCIBLE PROTEIN 1 IN MURINE EMBRYONIC STEM CELLS BIOLOGY

Jenny Andrea Arevalo Romero, Marilene Hohmuth Lopes
Departament of Cell and Developmental Biology, Institute of Biomedical Sciences, Sao Paulo, Brazil

Stress-inducible protein 1 (STI1) is a ubiquitous protein which present wide functional versatility since it is able to mediate distinct processes involved in neural plasticity such as cell proliferation, memory consolidation and self-renewal of neural stem cells, acting in association with its principal ligand, the prion protein (PrPc). Interestingly, STI1 has been found abundantly expressed since early stages of murine development (embryonic day, E8) to adulthood, suggesting that STI1 could play a role on mammalian development. Interestingly, previous results from our group shows that STI1 constitutive knockout embryos presenting remarkable deficiency and retarded growth, since the majority remain in blastocyst stage and only few embryos reaching E10 stage. Taken together, these recent findings might suggest STI1 as an essential molecule able to regulate self-renewal or differentiation on the early mammalian development. In fact, the best model to characterize the function of STI1 during early development stages is throughout the derivation of embryonic stem cells from the inner cell mass of pre-implantation blastocysts of knockout mice to STI1 and compare with wild-type ones. Since we are still establishing the ideal conditions to embryonic stem cells derivation, we decide perform knockdown of STI1 protein from an embryonic stem cell line ES-E14TG2A, as an alternative method to evaluate the function of STI1. Thus, this study is an original proposal which aims to evaluate the function of STI1 molecule on developmental biology of mammals.

F-2314

DIFFERENTIAL LOCALIZATION AND HIGH EXPRESSION OF SURVIVIN SPLICE VARIANTS IN HUMAN EMBRYONIC STEM CELLS BUT NOT IN DIFFERENTIATED CELLS IMPLICATE A ROLE FOR SURVIVIN IN PLURIPOTENCY

Amber N. Baer, Amanda Klar, Christopher S. Navara
Cell and Molecular Biology, University of Texas San Antonio, San Antonio, TX, United States

The BIRC5 gene encodes the oncofetal protein Survivin, as well as four additional splice variants (Ex3, 2B, 3B and 2α). Survivin, an inhibitor of apoptosis, is also a chromosomal passenger protein (CPP). Previous results have demonstrated that Survivin is expressed at high levels in embryonic stem cells (ESCs) and inhibition of Survivin function results in apoptosis, however these studies have not investigated the other four splice variants. In this study, we demonstrate that all variants are expressed at significantly higher levels in human ESCs than in differentiated cells. We examined the subcellular localization of the three most highly expressed variants. Survivin displayed canonical CPP localization in mitotic cells and cytoplasmic localization in interphase cells. In contrast, Survivin-Ex3 and -2B did not localize as a CPP; Ex3 was found constitutively in the nucleus while Survivin-2B was distributed along the chromosomes during mitosis and also to the mitotic spindle poles. We used inducible shRNA against Survivin to inhibit expression in a titratable fashion. Using this system, we reduced the mRNA levels of these three variants to approx. 40%, resulting in a concomitant reduction of Oct4, and Nanog mRNA, suggesting a role for the Survivin variants in pluripotency.

F-2315

TCF7L1 REPRESSES KEY PRIMITIVE STREAK REGULATORS IN HUMAN EMBRYONIC STEM CELLS

Robbie Sierra
University of California, Irvine, Irvine, CA, United States

Human embryonic stem cells (hESCs) are thought to be the in vitro representation of an epiblast-stage pluripotent cell-type. In the embryo, pluripotent epiblast cells initiate germ layer formation during gastrulation, which can be identified by a structure called the primitive streak. Like epiblast cells at this stage of development, hESCs use signaling pathway's as queues to direct germ layer differentiation. Mechanistically speaking, we want to understand how signaling pathway's influence hESC fate decisions. Specifically, our research is focused on a family of four transcription factors called LEF/TCFs, which are downstream transcription factor effectors of the Wnt signaling pathway. We have profiled the expression of LEF/TCFs in hESCs and discovered that TCF7L1 is the most highly expressed family member in hESCs. Using TCF7L1 loss-of-function experiments, in conjunction with microarray analysis, we have determined that TCF7L1 represses key primitive streak/gastrulation regulatory genes in hESCs. We therefore propose that TCF7L1 maintains hESC pluripotency by repressing genes involved in primitive streak/gastrulation.

F-2316

THE WNT SIGNALING PATHWAY INACTIVATES TCF7L1/TCF3 IN EMBRYONIC STEM CELLS AND BREAST CANCER

Brian R. Shy, Chun-I Wu, Jenny Zhang, Bradley J. Merrill
Biochemistry & Molecular Genetics, University of Illinois at Chicago, Chicago, IL, United States

Tcf7l1 (previously named Tcf3) and its interaction with beta-catenin are required for mammalian development. Conventionally, beta-catenin is thought to convert the Tcf/Lef transcription factors (Tcf7, Tcf7l1, Tcf7l2, Lef1) into transcriptional activators. However, the regulation of Tcf7l1 is unique. We find that interaction with beta-catenin leads to Tcf7l1 inactivation, and Tcf7l1-transactivator activity is not necessary for mouse embryogenesis or viability through adulthood. This inactivation of Tcf7l1 appears to be the primary role of nuclear beta-catenin in embryonic stem cells. The mechanism of inactivation involves multiple aspects of post-translational modification, proteasome-mediated degradation, and reduction of chromatin occupancy.

In addition to elucidating the mechanism by which Tcf7l1 is inactivated in embryonic stem cells, this work also extends the findings to poorly differentiated breast cancers. Both embryonic stem cells and poorly differentiated breast cancers exhibit high levels of Tcf7l1 mRNA. In addition, they both appear to require some level of constitutive Wnt pathway activation. Wnts cause the accumulation of nuclear beta-catenin which is then available to interact with the Tcf/Lef transcription factors. In human breast cancer tumors, nuclear and cytoplasmic beta-catenin protein is associated with low nuclear Tcf7l1 protein. In breast cancer cell lines, Tcf7l1 is inactivated and degraded in response to Wnt pathway stimulation. We suggest that like embryonic stem cells, the role of beta-catenin may be to inactivate Tcf7l1 in poorly differentiated breast cancers. These forms of breast cancer are aggressive and lack effective treatment options. If Wnt pathway activation is required to reduce Tcf7l1 repression, then targeting the enzymes or interactions responsible could provide a new therapeutic approach to attack these cancers.

F-2317

IDENTIFICATION OF TRANSLATIONAL NETWORKS ORCHESTRATING PLURIPOTENT CELL FATE THROUGH EIF4E REGULATION

Henia Darr, Dung-Fang Lee, Christoph Schaniel, Ihor Lemischka
Developmental & Regenerative Biology, Mount Sinai School of Medicine, NYC, NY, United States

Pluripotent cells hold a great promise for potential use in cell replacement therapy. However, prior to clinical use, it is important to understand mechanisms orchestrating pluripotency. Most research in the field has focused on characterization of the pluripotent cell's transcriptome and epigenome. However, RNA is not simply an intermediate enabling flow of information from genome to a protein, but rather is a regulatory core on which multiple proteins operate from transcription, through translation, until degradation. This is still an unexplored regulatory level of pluripotency. To understand the role of post-transcriptional gene regulation in the pluripotent state, we examined pathways involved in regulating RNA fate and identified numerous RNA binding proteins (RBPs) whose down-regulation impairs mouse embryonic stem (ES) cell pluripotency. One of them, Neuroguidin, regulates eIF4E dependant translation initiation. Due to the immense importance of eIF4E regulation in processes such as malignant transformation, development and cell fate determination, the involvement of eIF4E regulation in pluripotency was further investigated. Analysis of additional genes controlling eIF4E activity revealed tight dependence of ES cell self-renewal on proper eIF4E regulation. Both genes regulating global levels of eIF4E availability in the cell (such as 4E-bp1) and genes regulating translation of specific transcripts (such as Cpeb) are crucial for maintaining mouse ES cell self-renewal. Current work focuses on elucidating the gene network whose translation is orchestrated by Cpeb in ES cells. In addition, specific roles of each of the eIF4E binding proteins regulating pluripotency will be examined. Ultimately, we wish to understand how the collection of transcripts in the cell is orchestrated through interactions with multiple RBPs to define pluripotency, and incorporate these findings with knowledge on the transcriptome and epigenome of pluripotent cells.

F-2321

WNT/β-CATENIN ACTIVITY IN STEM CELLS MEDIATED BY DEGRADATION OF TCF7L1 PROTEIN

CHUN-I WU, Brian R. Shy, Bradley J. Merrill
Univeristy of Illinois at Chicago, Chicago, IL, United States

Wnt/β-catenin signaling plays important functions for self renewal and lineage specification of stem cells, morphogenesis, and tumor formation. Interestingly, Wnt/β-catenin stimulates self renewal of mouse ES cells and mesoderm specification of human ES cells. Wnt/β-catenin signaling functions by stabilizing β-catenin, which subsequently binds to nuclear Tcf/Lef transcription factors. In classic models of the pathway, β-catenin-Tcf/Lef complexes act as transcriptional activators by recruiting core machinery or epigenetic modifiers to Tcf/Lef target genes. Here we show mouse Tcf7l1 (formerly named Tcf3) mediates a distinct and new form of regulation of the pathway, which is independent of transactivator activity of Tcf7l1-β-catenin complexes. Instead of switching Tcf7l1 into a transactivator, β-catenin binding reduces Tcf7l1 protein levels, an effect sufficient to relieve repression of target genes. Exper-

iments using mouse ES cells show that recombinant Wnt3a, GSK3-inhibition, or simply increasing β-catenin levels were all sufficient to reduce Tcf7l1 protein independent of mRNA levels. The effect did not occur in Tcf7l1ΔN/ΔN ES cells, which lack nine Tcf7l1 residues necessary for binding to β-catenin. Thus, β-catenin binding to Tcf7l1 is necessary and sufficient for destabilizing Tcf7l1 protein in stem cells. Interestingly, various morphogenetic defects and lethality of Tcf7l1ΔN/ΔN knockin mice was rescued by reducing the amount of Tcf7l1 protein, as Tcf7l1-/ΔN mice displayed normal embryonic development and viability through adulthood. Thus, a Tcf7l1-β-catenin transactivator activity is not needed in mice. These mouse genetic experiments demonstrate that simply reducing the levels of Tcf7l1 effectively replaces the need for Tcf7l1-β-catenin interaction. This new biochemical activity of Wnt/β-catenin signaling helps elucidate mechanisms underlying the pro-self-renewal effects of Wnt signaling on mouse ES cells, which express high levels of Tcf7l1.

F-2322

IDENTIFICATION OF 5` AND 3` END REGION IN PORCINE XIST AND METHYLATION STATUS ANALYSIS OF PORCINE XIST CPG SITES IN PIGS

Jae Yeon Hwang[1], Eun-Bae Kim[2], **Chang-Kyu Lee**[1]

[1]Department of Agricultural Biotechnology, Animal Biotechnology Major, Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, Korea, Republic of, [2]Department of Food Science and Technology, University of California, Davis, California, USA, Davis, CA, United States

X-chromosome inactivation (XCI) is occurred in female mammal to match the expression levels of the genes in X chromosome between the male and female. The process is induced by X-chromosome inactivation specific transcript, *XIST/Xist*, in female eutherian during early embryo development. This long, non-coding RNA has been suggested essential gene for initiating XCI and these days, the gene is applied in various research fields such as stem cell research and pre-implantation embryo development. Although the importance of *XIST* has been highlighted, only tiny partial coding regions of the gene has been identified in pig. Previously we performed BLAST search and RT-PCR analysis to identify whole porcine *XIST* coding regions but its transcription starting site (TSS) and 3` end region was still undefined. So in this study, we confirmed complete sequence of the porcine *XIST* gene with 5` and 3` RACE PCR. The result was validated using RNA-Seq alignment process. The results showed that the identified TSS and 3` end region were matched to our previously performed BLAST search and RNA-Seq alignment results. The methylation patterns of *XIST/Xist* promoter regions were applied to analyze pluripotent status in naïve and primed stem cells. So, to suggest candidate porcine *XIST* CpG sites and confirm the methylation status in both sexes, we searched CpG sites in ± 2Kb region from the identified TSS, and analyzed the methylation status in male and female embryonic fibroblast. Seventy-eight CG dinucleotides were present in the region and sixty-seven of these dinucleotides were regarded to be candidate CpG sites. They were distributed compactly within two regions: -284 to +53 (13) and +285 to +1727 (54) from the TSS. A total of 67 CpG sites were 49.85% and 96.26% methylated in female and male, respectively. The results are considered to support the analysis of XCI in pigs and could be applied to stem cell and embryo development research in the pig. With the results, epigenetic differences on X-chromosomes and CpG sites of porcine *XIST* in naïve and primed pig stem cells will be analyzed. Also, XCI process in pigs will be confirmed in early embryo development and stem cell which has similar characters to inner cell mass in blastocyst further.

F-2324

GLOBAL ANALYSIS OF STABILITY OF UNDIFFERENTIATED STATE IN HUMAN PLURIPOTENT STEM CELLS

Daihachiro Tomotsune[1], Akimi Mogi[1], Fengming Yue[1], Susumu Yoshie[1], Shoko Masuda[1], Ayana Sakurai[1], Daiki Fujita[1], Mika Nagai Morisaki[2], Sakiko Shirasawa Takizawa[2], Tadayuki Yokoyama[2], Katsunori Sasaki[1]

[1]Histology and Embryology, Shinshu University School of Medicine, Matsumoto, Nagano, Japan, [2]Bourbon Institutes of Health, Matsumoto, Nagano, Japan

Maintenance of undifferentiated state is a critical requirement for regenerative medicine using pluripotent stem cells. The undifferentiated state in pluripotent stem cells is achieved by the actions of pluripotency master regulators such as Oct4, Sox2 and Nanog, and maintained by epigenetic factors such as polycomb group. Most studies focusing on the undifferentiated state have been carried out using experimental systems which induce differentiation of pluripotent stem cells into the particular (expected) cell type. However, although pluripotent stem cells including ES cells and iPS cells are well known to differentiate spontaneously, the differentiation mechanism in this natural culture condition is poorly understood. In this study, we produced the spontaneous differentiation by removing MEF cells, and then analyzed global changes of gene expression during the spontaneous differentiation (collapse of undifferentiated state) using DNA microarray. As results of the microarray analysis using four human pluripotent stem cell lines, human ES cell: khES1 (Kyoto), khES2 (Kyoto), H1 (Wisconsin) and human iPS cell: 253G1 (Kyoto), we found that some genes were commonly up- or down-regulated during the collapse of undifferentiated state. These genes included not only expected genes such as Nanog, but also unexpected genes, such as adenylate kinase 2. These genes are assumed to induce collapse of undifferentiated state or to be sensitive to the collapsing event, so they were called as collapse related genes. In addition, the four cell lines showed the different tendency to differentiation in our daily experiment. DNA microarray results indicated that some genes were commonly expressed in the cell lines with similar tendency of differentiation. These similar cell lines showed some similarities in the gene expression patterns during the collapse of undifferentiated state. Furthermore, we analyzed the role of polycomb group during the collapse of undifferentiated state by ChIP-seq and so on, in order to reveal relationship between the collapse related genes and epigenetic regulation.

F-2325

MAINTENANCE OF HUMAN EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS UNDER STABLE CLONOGENIC CONDITIONS WITH SMALL MOLECULES

Ludovic Zimmerlin, Karan Verma, Tea Soon Park, Lynette Naler, Elias T. Zambidis
Pediatric Oncology, Johns Hopkins University, Baltimore, MD, United States

Recent studies have highlighted dissimilarities between conventional human pluripotent stem cell (PSC) cultures and mouse embryonic stem cells (mESC). Human and mouse PSC are morphologically distinct, and exhibit several phenotypic and functional discrepancies, including the molecular pathways sustaining their self-renewal. While LIF-supported mESC utilize JAK/STAT signaling and can grow clonally, standard human PSC maintenance requires exogenous FGF2 for activation of the MEK/ERK/Activin axis, and passaging via aggregates after mild enzymatic digestion due to limited clonal survival. Various strategies have been proposed either to alleviate these constraints (e.g., using surrogate feeders to circumvent FGF2 supplementation, or addition of survival-enhancing small molecules such as ROCK inhibitor to tolerate single cell dissociation), or switching human PSC cultures to mESC-like metastable states. Here, we introduce novel culture conditions that allow human PSC to expand clonally and retain pluripotency via modulation of protein kinase activity. The human PSC lines tested in this study included the human ESC line H9, and several cord blood (CB)-derived and fibroblast-derived human induced pluripotent stem cell (iPSC) lines. Standard culture conditions containing supplemented FGF2 were transferred to a modified N2B27-based medium supplemented with LIF, and a cocktail of small molecules regulating protein kinase activity. Single-cell passaging was performed via enzymatic digestion (Accutase), and growth kinetics and cellular viability were assessed by cell counts using Trypan blue. After a short initial adaptation step (2-4 passages), all tested cell lines reached a stable phenotype with robust growth kinetics and uniform morphology for up to 20 passages. Flow cytometry analysis showed homogenous expression of SSEA-4, TRA-1-60 and TRA-1-81 in all FGF-free cultures and immmunofluorescent stainings revealed uniform retention of NANOG and E-cadherin expression throughout small compact colonies. Expression of pluripotency-associated markers was also confirmed by Q-RT-PCR (Oct-4, Nanog, Rex1) in converted CB-iPSC, at levels matching or superior to standard FGF-supplemented cultures. Pluripotency potential was validated by teratoma formation in NOD/Shi-scid/IL-2Rγnull mice. In summary, these novel culture conditions can robustly sustain clonal growth of human PSC without requirement for exogenous FGF2. We are currently specifying the identity of these converted lines and comparing them to their parental FGF2-dependent lines by de-

termining the molecular pathways involved for their self-renewal, and their capacity for gene targeting by homologous recombination.

F-2326

AN EXPANDED NANOG INTERACTOME FOR STEM CELL PLURIPOTENCY AND SOMATIC CELL REPROGRAMMING

Junjun Ding[1], Francesco Faiola Faiola[1], Yael Costa[3], Thorold Theunissen[3], Huilei Xu[2], Miguel Fidalgo[1], Arven Saunders[1], Avi Ma'ayan[2], José Silva[3], Jianlong Wang[1]

[A]Department of Developmental and Regenerative Biology, Black Family Stem Cell Institute, [B]Department of Pharmacology and Systems Therapeutics, [1]mount sinai school of medicine, New York, NY, United States, [2]Wellcome Trust Centre for Stem Cell Research and Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom

Nanog are well-known transcription factors that play fundamental roles in stem cell self-renewal, pluripotency and somatic cell reprogramming. However, limited information is available on Nanog associated protein complexes and their intrinsic protein-protein interactions that dictate the critical regulatory activities of Nanog. Here we employed an improved affinity purification approach combined with mass spectrometry to purify Nanog associated protein complexes in mouse embryonic stem cells (mESCs), and discovered 119 Nanog interacting proteins and many of them are novel Nanog partners important for self-renewal and pluripotency of mESCs. Notably, we found that Nanog are associated with multiple chromatin modifying complexes, such as NuRD complex, Polycomb complex, Lsd1 complex, MLL1 complex, Sin3 complex and Ten eleven translocation (Tet) family methylcytosine hydroxylase Tet1 and Tet2, with documented as well as newly proved functional significance in stem cell maintenance and somatic cell reprogramming. We will present an expanded Nanog interactome together with the physical and functional validation of the interactome. Our study establishes a solid biochemical basis for genetic and epigenetic regulation of stem cell pluripotency and provides a framework for further dissecting the molecular mechanism underlying Nanog's function in pluripotency and reprogramming.

F-2327

EMBRYONIC STEM CELL GENOMIC INSTABILITY RESULTING FROM CULTURE PASSAGES MAY BE A MECHANISM OF ADAPTATION AND PLURIPOTENCY MAINTENANCE

CLARA I. ESTEBAN-PEREZ[1], HAROLD H. MORENO-ORTIZ[2], CAROLINA LUCENA[3], DWAYNE WISE[1], NANCY REICHERT[1]

[1]BIOLOGICAL SCIENCES DEPARTMENT, MISSISSIPPI STATE UNIVERSITY, STARKVILLE, MS, United States, [A]REPRODUCTIVE GENETICS, [B]STEM CELLS UNIT, [2]FERTILITY AND STERILITY COLOMBIAN CENTER-CECOLFES, BOGOTA, Colombia

Embryonic stem (ES) cells have the ability to maintain pluripotency and self-renewal during in vitro maintenance, which is a key to their clinical applications. ES cell quality has been widely evaluated through determination of the specific genetic and epigenetic profiles. The hypothesis of this study was that genetic stability in repetitive sequences located near key genes involved in pluripotency, self-renewal, differentiation, chromatin assembly, and imprinting could be a signal for adaptation of the ES cell in vitro. Instability in specific repetitive sequences is present and increases during ES cell passages. ES cells displayed significant mean frequencies of instability in twelve markers out of 64 related to pluripotency (OCT4, D1S551), early differentiation (G60405, D18S63, and D1S468), chromatin assembly (D22S447, D6S2252, D10S529, and HISTB2), and imprinting (GRB10-prom, D2S144, and IGF2-prom). Interestingly, instability was distinct between H1 and H7 ES cell lines. In summary, these results suggest that instability in tandem repeat sequences located near early embryonic developmental genes is associated with failure of ES cell pluripotency and self-renewal maintenance over consecutive culture passages. These results suggest that instability determination is a potential indicator of gene deregulation and epigenetic modification that involves chromatin modification and imprinting establishment during ES cell cultures. Finally, instability in

specific genes could be a signal that contributes to adaptation of ES cells to in vitro culture or could be the switch that initiates early cell specialization in vitro.

F-2328

STATISTICAL MECHANICS OF EMBRYONIC STEM CELL HETEROGENEITY

Ben MacArthur[1], Ihor Lemischka[2]
[1]Faculty of Medicine, University of Southampton, Southampton, United Kingdom, [2]Mount Sinai School of Medicine, New York, NY, United States

A number of recent studies using high-throughput single cell gene expression profiling have uncovered a surprising degree of cell-to-cell variability within apparently functionally homogeneous embryonic or induced pluripotent stem cell populations. Collectively, these reports suggest a statistical view of pluripotency in which both deterministic and stochastic mechanisms play key parts. However, the molecular basis for this variability is not fully known. Here, using an integrated approach - that includes high-throughput single-cell transcriptional profiling, bioinformatics and mathematical modelling - we will examine the role that feedback loops in the ESC transcriptional regulatory circuitry have in regulating population heterogeneity. We will outline a statistical mechanics view in which heterogeneity, appropriately defined, is an essential feature of pluripotent stem cell populations.

F-2331

B-CELL RECEPTOR ASSOCIATED PROTEIN 31 REGULATES THE SELF-RENEWAL AND PLURIPOTENCY OF HUMAN PLURIPOTENT STEM CELLS VIA THE REGULATION OF APOPTOSIS ON THE CELL SURFACE

Won-tae Kim, Hong Seo Choi, Hyun Min Lee, Chun Jeih Ryu
Sejong university, Seoul, Korea, Republic of

B-cell
receptor associated protein 31 (BAP31), a resident integral protein of the
endoplasmic reticulum (ER) membrane, regulates the export of secreted membrane protein from the ER to the downstream secretory pathway. In the previous study, we generated monoclonal antibodys (MAbs) against the surface molecules on undifferentiated human embryonic stem cells (hESCs) by using a modified decoy immunization strategy. Of these, 297-D4 bound to human pluripotent stem cells but not to human differentiated primary cells, mouse embryonic stem cells, and mouse embryonic fibroblasts. 297-D4 antigen expression was localized to the undifferentiated and pluripotent hESCs and rapidly downregulated during early differentiation. 297-D4 antigen, an approximately 30 kDa cell surface protein, was identified as BAP31 by peptide mass fingerprinting and western blot analysis. 297-D4 binding reactivity to BAP31 was further confirmed by ectopic expression of BAP31 gene. Thus, cell surface-expressed BAP31 was involved in the undifferentiated and pluripotent
state of hESCs. siRNA-mediated depletion of BAP31 reduced the number of alkaline phosphatase-positive colonies in hESCs and led to a decrease in the expression of pluripotency-associated transcription factors Oct4, Nanog and Sox2, suggesting that BAP31 is essential for hESC self-renewal and pluripotency. BAP31 depletion caused an increase in the expression of differentiation genes associated with the early development of three germ layers and induced a substantial level of apoptosis in hESCs. Analysis of apoptosis-related genes revealed that BAP31 depletion induced the expression of Bid but not Puma and Noxa, suggesting that cell surface-expressed BAP31 may be responsible for the regulation of extrinsic apoptotic pathway. Induction of apoptosis by ER stress and serum starvation resulted in decreased expression of cell surface-expressed BAP31, suggesting that cell surface-expressed BAP31 is closely ssociated with anti-apoptotic potential. Finally, analysis of signaling molecules further
revealed that phosphorylation of AKT was decreased in BAP31-depleted hESCs. These findings provide for the first time mechanistic insights how BAP31 regulates hESC self-renewal and pluripotency on the cell surface.

F-2332

PATTERNS OF DISTINCT MITOCHONDRIAL METABOLISM IN HUMAN PLURIPOTENT STEM CELLS BEFORE AND DURING DIFFERENTIATION

Sandra L. Escobar[1], Tara TeSlaa[2], Jin Zhang[3], Sarah Imam[3], Esther Nuebel[4], Mahta Nili[3], Kiyoko Miyata[3], Cindy S. Malone[1], Carla Koehler[5], Michael A. Teitell[6]

[1]Deparment of Biology, California State University, Northridge, Northridge, CA, United States, [A]Molecular Biology Institute, [B]Department of Pathology and Laboratory Medicine, [C]Department of Chemisty and Biochemistry, [D]Department of Chemistry and Biochemistry, [E]Jonsson Comprehensive Cancer Center, California NanoSystems Institute, [2]University of California, Los Angeles, Los Angeles, CA, United States

Human pluripotent stem cells (hPSCs) are defined by their ability to self renew and differentiate into all three germ layers. Their potential for robust cell division requires a distinct metabolic profile compared to less proliferative differentiated cells. The tricarboxylic acid (TCA) cycle in the mitochondria directs electrons into the respiratory chain, provides biosynthetic precursors for anabolic metabolism, and maintains levels of important enzyme cofactors. The focus of study here is in characterizing hPSC metabolism and its role in regulating hPSC self-renewal and differentiation. We have shown that hPSCs contain a fragmented mitochondrial network. In addition, hPSCs are dependent on hydrolyzing the ATP produced by glycolysis to maintain the mitochondrial inner membrane potential and avoid apoptosis. We show that uncoupled protein 2 (UCP2) is highly expressed in hPSCs and promotes glycolysis. Expression of UCP2 decreases with differentiation, and maintaining the hPSC level of UCP2 expression inhibits differentiation. UCP2 inhibits glucose oxidation by shunting pyruvate away from the mitochondrial TCA cycle. However, hPSCs must maintain TCA cycle intermediates that are required for essential enzymes and anabolic pathways. We generated shRNA knockdown of selected TCA cycle enzymes to investigate the effect on hPSCs before and during differentiation. Additionally, we have designed experimental models to examine metabolic pathways with potentially significant roles in the epigenetic regulation of hPSC self-renewal and differentiation. Our goal is to determine how the TCA cycle supports distinct biosynthetic requirements in hPSCs and their differentiated progeny. An improved understanding of metabolic changes that promote distinct cell stages will provide new insights into mechanisms that influence cellular crosstalk between metabolic intermediates and epigenetic factors during hPSC growth and differentiation. Our work will also help in establishing safe conditions for utilizing hPSCs in potential therapeutic applications and as new models of disease.

Embryonic Stem Cell Differentiation

F-2333

EFFICIENT HEMATOPOIETIC DIFFERENTIATION OF COMMON MARMOSET EMBRYONIC STEM CELLS BY THE INHIBITION OF THEIR SELF-RENEWAL PATHWAY

Takenobu Nii, Tomotoshi Marumoto, Hirotaka Kawano, Saori Yamaguchi, Yoko Nagai, Jiyuan Liao, Michiyo Okada, Yoshie Miura, Kenzaburo Tani

Department of Molecular Genetics, Division of Molecular and Clinical Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan

Various kinds of functional cells differentiated from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have recently been developed for use in human regenerative medicine. However, the safety and efficacy of ESC/iPSC-based therapies must be carefully evaluated prior to clinical application, using reliable animal disease models. The common marmoset (CM, Callithrix jacchus) is known to be a suitable preclinical model for clinical translation studies, and CM ESCs have already been established.

Understanding the self-renewal pathways in ESCs is crucial for the development of improved technology to culture and differentiate them into functional cells of potential therapeutic use. Previous in vitro studies demonstrated that basic fibroblast growth factor (bFGF), which activates the PI3K-AKT and MEK-ERK pathways, was able to maintain the pluripotency and self-renewal capacity of human ESCs, whereas leukemia inhibitory factor (LIF), which activates the JAK-STAT3 and PI3K-AKT pathways, maintained the same abilities in mouse ESCs. However, the specific growth

factors required for CM ESC/iPSC culture have not been determined. In this study, we investigated the abilities of bFGF and LIF to maintain self-renewal of CM ESCs/iPSCs in culture. CM ESCs/iPSCs were shown to express both bFGF receptors (FGFR1, FGFR2, FGFR3 and FGFR4) and LIF receptors (LIFR and gp130). It appeared that addition of bFGF resulted in the promotion of self-renewal in CM ESCs, while addition of LIF had no effect on it. Moreover, this effect of bFGF on the promotion of CM ESC self-renewal was due to the activation of both PI3K-AKT and MEK-ERK pathways.
We have also found that CM ESCs are differentiated into hematopoietic lineages by embryoid body (EB) formation in the presence of hematopoietic cytokines, but the rate of hematopoietic differentiation was low (less than 5%). In this study, we hypothesized that transient inhibition of ESC self-renewal pathway during EB formation would improve hematopoietic differentiation of CM ESCs. To test this hypothesis, we performed EB formation assay with or without PI3K inhibitor (LY294002). We found that treatment of LY294002 rapidly decreased the population of NANOG+/OCT4+ cells representing undifferentiated ESCs. Moreover, the populations of both CD34+/FLK1+ cells (hematoendothelial cells) and CD34+/c-Kit+ cells (hematopoietic progenitor cells) were significantly increased in the presence of LY294002. The point in time when populations of both CD34+/FLK1+ and CD34+/c-Kit+ cells were maximal was day8 in LY294002 treated EBs, which was 8 days earlier than that in untreated controls. Furthermore, the cells from day8-EBs formed hematopoietic colonies in colony forming unit (CFU) assay. These results indicate that hematopoietic differentiation was promoted by transient LY294002 treatment (4days) in the process of EB formation.
Overall, the inhibition of self-renewal pathway in the process of EB formation could be a more efficient method to induce hematopoietic differentiation of CM ESCs. Furthermore, our findings might contribute to the development of the method for in vitro expansion of hematopoietic stem/progenitor cells (HSCs/HPCs) as well as the preclinical studies to test HSCs/HPCs from CM ESCs/iPSCs for the future ESC/iPSC-based human regenerative medicine.

F-2334

EFFICIENT ERYTHROMYELOID AND LYMPHOID DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS IN COCULTURE WITH MURINE STROMAL CELLS

Everton de Brito Oliveira Costa[1], Maristela Delgado Orellana[4], Danielle Aparecida Rosa de Magalhães[4], Lilian Figueiredo Moreira[4], Virgínia Mara de Deus Wagatsuma[4], Ricardo Bonfim Silva[2], Simone Kashima Haddad[4], Aparecida Maria Fontes[2], Dimas Tadeu Covas[3]

[A]Clinical Oncology, Stem Cells and Cell Therapy, [B]Genetics, [C]Medical School of Ribeirao Preto, [1]University of Sao Paulo, Ribeirao Preto, Brazil, [2]Blood Center of Ribeirao Preto, National Institute of Science and Technology in Stem Cells and Cell Therapy, Ribeirao Preto, Brazil

Hematopoiesis has been well described in murine models in the recent decades. But studies that have characterized hematopoiesis in humans are still scarce. Also, methods to obtain and isolate hematopoietic cells have great impact on current clinical practice in Hematology. The use of human embryonic stem cells (hESCs) has generated new perspectives in studying hematopoiesis as an attempt to mimic what naturally occurs during embryonic development, as much as for clinical application of hematopoietic cells obtained from the differentiation of these cells. However, although several studies have shown the production of hESCs-derived hematopoietic cells, the protocols have generated varied quantities of cells with low efficiency and functional properties of primitive stem cells. Thus, we sought to establish a new model for hESC-H1 differentiation in hematopoietic progenitor cells so that they can be better characterized and obtained more efficiently. For this purpose, we developed an in vitro differentiation system based on co-culture of hESC-H1 line, maintained in mTeSR™1 medium (STEMCELL Technologies, Vancouver, Canada), with murine stromal cells in differentiation medium supplemented with low concentrations of cytokines and hematopoietic growth factors. As result, the development of this study allowed the establishment of a method to generate a mixed population of cells enriched in hematopoietic progenitor cells positive for the marker CD45 (71+17%), which proved to be co-expressed with other hematopoietic markers (CD31 [62+16%], CD43 [67+17%], CD71 [55,2±18,8%] and CD38 [45±7,21%]). We have obtained cells of the erythroid and myeloid lineage at different stages of maturation evidenced by specific markers (235a [9±2%], CD14 [12,7±4%], CD15

[35,7±11,7%], CD16 [20,3±7,57%]), with viability greater than 70% during more than 30 days. It was demonstrated that these cells expressed genes related to primitive and definitive hematopoiesis (PTPRC, PECAM1, RUNX1, TAL1, LMO2, PROM1, CD34 and NOTCH1). These cells also showed in vitro clonogenic potential of 1/574 plated cells from the total cells obtained of the differentiation without sorting for specific population and they were able to produce CFU-GEMM, BFU-E and CFU-GM in MethoCult® H4034 Optimum (STEMCELL Technologies, Vancouver, Canada). Interestingly, it was observed that there was a 10 fold improvement of GM-CFC colonies formation in methylcellulose after the addition of TGF-B1 to the differentiation medium demonstrating an important role of this molecule in hematopoiesis. On the other hand, the co-culture of hESC-H1 with murine stromal cells without hematopoietic cytokines and growth factors produced detectable lymphoid cells positive for CD4(33,9%), CD3(27,2%), CD45(33,9%), CXCR4(29,7%), CD117(20%) markers, and negative for CD8 marker. These results demonstrated the possibility to generate erythromyeloid and lymphoid cells by adapting this differentiation protocol and provide insights into ontogeny of hematopoietic lineages both in ES cell-derived cultures and during normal development. Moreover, the observations of the predominance of lineage-specific precursors during differentiation open up alternatives to select specific cell populations to direct the differentiation to specific mature hematopoietic lineages and also to obtain hematopoietic stem cells in vitro for future clinical applications.

F-2335

SPECIFYING HEMATOPOIETIC STEM CELLS FROM HUMAN PLURIPOTENT CELLS USING DEFINED TRANSCRIPTION FACTORS

Sergei Doulatov[1], Thuy D. Vo[1], Hu Li[2], James J. Collins[2], George Q. Daley[1]

[1]Hematology/Oncology, Boston Children's Hospital, Boston, MA, United States, [2]Howard Hughes Medical Institute, Department of Biomedical Engineering and Center for BioDynamics, Boston University, Boston, MA, United States

Hematopoietic stem cells (HSCs) are defined by the capacity for self-renewal and multilineage differentiation. By contrast, primitive progenitors lack self-renewal capacity and are restricted to myeloid and erythroid lineages. Differentiation of embryonic or induced-pluripotent stem cells (iPSCs) to blood generates predominantly primitive progenitors, hampering their use in regenerative medicine. Induction of HoxB4 instructs HSC fate from murine yolk sac and embryoid body (EB)-derived primitive progenitors, suggesting that the transcription factors (TF) that maintain HSC identity can reprogram primitive progenitors to HSC, but HoxB4 has not functioned similarly in human cells. To identify factors that function in the context of human pluripotent stem cells, we developed a screen for self-renewal potential in which EB-derived CD34+CD45+ primitive hematopoietic progenitors (HPCs) were cultured for a week before plating into colony assays. HPCs transduced with a library of 10 HSC TFs routinely gave rise to colonies in this screen, while controls failed to demonstrate replating capacity. This activity was winnowed to a combination of 5 TFs (5F), which reliably converted primitive myeloid HPCs into multilineage progenitors in vitro. CD34+45+ HPCs transduced with 5F produced a heterogeneous population of hematopoietic derivatives including rare phenotypic HSCs (Lin-CD34+CD38-CD90+), CD34+CD38+ progenitors, and mature myeloid and erythroid cells. Transplantation of these cells into NSG mice consistently enabled low-levels of human engraftment. Gene expression comparison of CD34+CD38- 5F cells with those isolated from EB and cord blood showed reactivation of HSC programs coupled with a repression of myeloid, cell cycle and metabolism genes characteristic of definitive HSCs. However, the magnitude of these gene expression changes in many cases was below the level of HSCs, suggesting that global reactivation of HSC genes may be opposed by a restrictive chromatin state in embryonic cells. Screens of additional factors are underway to expand the HSC potential of reprogrammed human progenitors derived from iPSCs.

F-2336

INABILITY TO INDUCE HOXA CLUSTER EXPRESSION COMPROMISES SELF-RENEWAL OF HESC-DERIVED HSPCS

Diana R. Dou[1], Arazin Minasian[1], Maria I. Sierra[1], Pamela Saarikoski[1], Sacha L. Prashad[1], Rajkumar Sasidharan[1], Mattias Magnusson[1], Jian Xu[2], Stuart H. Orkin[2], Jerome A. Zack[1], Gay M. Crooks[1], Zoran Galic[1], Hanna K.A. Mikkola[1]

[1]Eli and Edythe Broad Stem Cell Research Center, University of California, Los Angeles, Los Angeles, CA, United States, [2]Division of Hematology/Oncology, Children's Hospital and Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, United States

The inability to derive functional hematopoietic stem cells (HSCs) in culture from pluripotent cells limits the therapeutic use of HSCs; however, the molecular defects compromising the in vitro generated hematopoietic stem/progenitor cells (HSPCs) are unknown. Using a two-step differentiation method in which human embryonic stem cells (hESCs) were first differentiated into embryoid bodies (EBs) and then CD34+ cells from hEBs were co-cultured on OP9M2 stroma (hEB-OP9), we were able to derive HSPCs with the human HSC immunophenotype (CD34+CD38-CD90+CD45+). Colony forming and co-culture assays demonstrated that the hEB-OP9 HSPCs were able to differentiate into myelo-erythroid lineages and T-cells; however, they were severely limited in their proliferative potential and ability to differentiate into B-cells as compared to their counterparts isolated from the human fetal liver (hFL), and failed to provide in vivo reconstitution in NSG mice.

To identify the basis of the self-renewal and differentiation defects, we performed microarray analysis to define gene expression differences between HSPCs from hEB-OP9, hFL, early 3-5 week placenta (hPL) and an earlier stage (day 15) of hESC differentiation (hEB). This analysis revealed establishment of the general hematopoietic transcription factor network (e.g. SCL, RUNX1, CMYB, HOXB4) demonstrating successful differentiation to HSPCs. Importantly, Spearman coefficients confirmed that hEB-OP9 CD90+HSPCs were more similar to hFL CD90+HSPCs (0.91) as compared to the developmentally immature hEB and hPL CD90+HSPCs (0.81 and 0.79, respectively). Moreover, hEB-OP9 HSPCs downregulated genes related to hemogenic endothelium development that were highly expressed in hEB and hPL HSPCs, while genes critical in HSPC function, including DNA repair machinery and chromatin modifiers, were upregulated to levels comparable to hFL-HSPCs.

However, a subgroup of hFL-HSPC genes remained very low in hEB-OP9 HSPCs, including the HOXA cluster genes (implicated in HSC self-renewal) and BCL11A (implicated in B-cell formation and globin switching). Interestingly, low levels of HOXA genes and BCL11A and poor proliferative potential were also observed in HSPCs from early placenta, suggesting these defects reflect an incomplete developmental maturation of hEB-OP9 HSPCs. In contrast to the HOXA cluster genes, which remained severely downregulated despite maturation on OP9M2 stroma, increasing time in OP9M2 co-culture improved BCL11A expression and also generated erythroid cells that elevated adult beta-globin mRNA expression, indicating that BCL11A expression and ability to induce adult globin mRNA are not definite blocks in hESC-HSPC developmental maturation. To assess the functional correlation of HOXA genes and BCL11A in HSPC proliferative potential and multipotency, we used shRNAs to target MLL (the regulator of the HOXA cluster), individual HOXA genes, or BCL11A in hFL-HSPCs. Knockdown of HOXA7 and MLL resulted in rapid loss of CD90+HSPCs followed by depletion of all CD34+ cells, while BCL11A silencing resulted in the loss of B-cells. These studies identify HOXA cluster and BCL11A as developmentally regulated genes essential for generating functional HSCs from pluripotent cells. However, whereas BCL11A expression can be induced with prolonged co-culture, the inability to induce HOXA cluster expression curtails developmental maturation of hEB-OP9 HSPCs and prevents the generation of self-renewing HSCs.

F-2337

EFFECTIVE GENERATION OF AN ENRICHED POPULATION OF HEPATIC PROGENITORS FROM HUMAN EMBRYONIC STEM CELLS USING LITHIUM CHLORIDE IN A DEFINED CULTURE MEDIUM

Jiyou Han, Jong Hyun Kim, Su Yoen An, Yu Jin Jang, Jeong Sang Son, Gyunggyu Lee, Ji Young Park, Jong-Hoon Kim
Life Science and Biotechnology, Laboratory of Stem Cell Research, Korea University, Seoul, Korea, Republic of

Human embryonic stem cells (hESCs) are an important future source of cell-based therapeutics for cirrhosis and fulminant liver failure. However, the scarcity of functional hepatocytes derived from hESCs for cell transplantation is

a great limitation. In this study, an efficient and rapid, three-step protocol for hepatic differentiation of hESCs is described. The protocol entails generation of functional hepatic progenitors directly from hESCs cultured in a defined culture medium with lithium chloride (LiCl), a potent inducer of hepatic progenitors. In the first step, hESC-derived embryoid bodies (EBs) cultured in the defined culture medium were induced to differentiate into endodermal hepatic cells much more effectively than those cultured in Dulbecco's modified Eagle's medium DMEM/F12. Addition of 10 mM LiCl enhanced further hepatic differentiation and elevated expression of the endodermal markers [alpha-fetoprotein (AFP) and forkhead box protein A2 (FOXA2)] and hepatic progenitor markers [Albumin (ALB), Hepatocyte Nuclear Factor 4α (HNF4α), and HEX], compared to the control group. Importantly, LiCl treatment caused endodermal cell enrichment by promoting apoptosis of non-hepatic cells, including undifferentiated cells. The hESC-derived hepatic progenitors had functional profiles similar to human fetal hepatocytes. Enriched hepatic progenitors were also induced to differentiate into mature hepatocytes with normal hepatocyte functions, such as glycogen accumulation and drug-metabolizing enzyme activity. In summary, our three-step protocol for generating hepatic progenitors improves the generation of hepatocytes from hESCs by controlling proliferation and expansion of a homogeneous population of hepatic progenitors. This research (Funding No. 2012028636) was supported by the Bio & Medical Technology Development Program of the National Research Foundation of Korea (NRF) and Ministry of Education, Science and Technology (MEST).

F-2338

DERIVATION OF METABOLICALLY FUNCTIONAL HEPATOCYTE LIKE CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Shinichiro Ogawa[1], James Surapisitchat[1], Mina Ogawa[1], Kim S. Sugamori[2], Shung Wang[2], Rebecca R. Laposa[2], Rachel F. Tyndale[2], Denis M. Grant[3], Gordon Keller[1]

[1]McEwen Centre for Regenerative Medicine, Toronto, ON, Canada, [A]Department of Pharmacology & Toxicology, Faculty of Medicine, [B]Department of Pharmacology & Toxicology, [2]University of Toronto, Toronto, ON, Canada

Human pluripotent stem cells (hPSCs) represent a novel source of hepatocytes for drug metabolism studies and cell-based therapy for the treatment of liver diseases. These applications are, however, dependent on the ability to generate mature metabolically functional cells from the hPSCs. The generation of functional cells from hPSCs is dependent on our ability to recapitulate critical aspects of embryonic liver development in the differentiation cultures. Efficient endoderm induction is the first key step in generating cells of the hepatic lineage from hPSCs. We have found that combined activin/nodal and wnt/β-catenin signaling is essential to induce efficient endoderm development as measured by C-KIT and CXCR-4 and the transcription factor SOX17+ in three dimensional embryoid body (EB) and monolayer cultures. The induction of highly enriched endoderm populations (>95% C-KIT+CXCR4+) is important for the generation of optimal hepatocyte-like cells. Once induced, an extended period of activin signaling is required to pattern the definitive endoderm to an appropriate stage for hepatic specification. The appropriately patterned endoderm is specified to a hepatic fate by the combination of BMP4 and bFGF. At this stage, the cells are cultured in the presence of HGF, Dex and OSM to generate a hepatic progenitor population that consists of 80%-95 % albumin positive cells that express HNF4α, produce glycogen and secrete albumin. To induce maturation, the hepatic progenitors are harvested from the monolayer and cultured for varying periods of time as aggregates. Beyond 40 days of differentiation, the aggregates consist of greater than 90% albumin+ cells, 60 % Asialoglycoprotein receptor-1 positive cells and express levels of albumin, G6Pase, and TAT comparable to those of found in the adult liver. Under appropriate conditions, the aggregated cells can be induced to mature to a stage which they express functional levels of phase I P450 genes including CYP1A2 and CYP3A4, and the phase II metabolic enzymes UGT1A1 and NAT1/2. Metabolic activity of the hPSC-derived hepatocyte-like cells, as measured by HPLC is similar to or higher than that found in primary human hepatocytes. Microarray analysis revealed that the hPSC-derived hepatocytes expressed the key P450 enzyme genes, as well as genes that encode proteins involved in many aspects of liver function including gluconeogenesis, glucose homeostasis, lipid metabolism and mitochondria biogenesis at levels comparable to or higher than the levels found in primary hepatocytes. Taken together, these findings show that efficient endoderm induction of hPSCs following by appropriate specification and maturation promotes the development of hepatocyte-like populations that display functional properties of primary human hepatocytes.

F-2341

RUNX1C EXPRESSION IDENTIFIES CD34-POSITIVE CELLS DIFFERENTIATED FROM HUMAN EMBRYONIC STEM CELLS WITH HEMATOPOIETIC PROGENITOR AND BONE MARROW HOMING ABILITY

Lisa Azzola[1], Katerina Vlahos[2], Elizabeth S. Ng[3], Claire E. Hirst[1], Vanta J. Jokubaitis[1], Qing C. Yu[1], Zhen Zhang[2], Brenda Williams[2], Magdaline Costa[1], David Haylock[2], Susan K. Nilsson[2], Edouard G. Stanley[3], **Andrew G. Elefanty**[3]
[1]Monash University, Clayton, Australia, [2]CSIRO, Clayton, Australia, [3]Murdoch Childrens Research Institute, Parkville, Australia

Transcription of the *RUNX1* gene, which plays an essential role in the regulation of definitive hematopoiesis, is controlled by proximal and distal promoters, leading to the generation of several isoforms. We targeted a green fluorescent protein (GFP) gene downstream of the RUNX1 distal promoter in hESCs, to generate heterozygous *RUNX1c*$^{GFP/w}$ hESCs and *RUNX1c*$^{GFP/GFP}$ lines in which both alleles of the RUNX1c gene were deleted. In *RUNX1c*$^{GFP/w}$ cells differentiated in defined medium, GFP accurately mirrored expression of the endogenous *RUNX1c* isoform and was observed after 8-10 days, following the sequential expression of CD34, CD43 and CD41. GFP expression was restricted to hematopoietic cells and GFP$^+$CD34$^+$ cells were highly enriched for hematopoietic progenitors and cells with the ability to home to the bone marrow of immunocompromised mice, although cells with long term repopulating ability were not identified. Examination of differentiating *RUNX1c*$^{GFP/GFP}$ cells revealed that RUNX1c was not required for the generation of hematopoietic colony forming cells during the first 14 days of hESC differentiation but that GFP$^+$CD34$^+$ cells from the *RUNX1c*$^{GFP/GFP}$ line homed less efficiently to the bone marrow. Although microarray analysis revealed that GFP$^+$CD34$^+$ cells were enriched for transcripts associated with hematopoietic stem and progenitors, comparison with the transcriptional profiles of umbilical cord blood CD34$^+$ cells, revealed that hESC-derived CD34$^+$ cells did not express HOXA cluster genes. These findings indicate that *RUNX1c* expression marks the subset of CD34$^+$ cells with progenitor and homing ability and suggests that differences in patterning might explain the failure of hESC derived blood cells to reconstitute hematopoiesis in transplantation models.

F-2342

MODELING PLACENTAL DEVELOPMENT USING HUMAN EMBRYONIC STEM CELLS

Mana Parast, Yingchun Li, Matteo Zita, Francesca Soncin, Anna Wakeland, Louise Laurent
Sanford Consortium for Regenerative Medicine, UCSD, La Jolla, CA, United States

The placenta is a transient organ, necessary for proper fetal development. Its main functional component is the trophoblast, which is derived from extraembronic ectoderm. Little is known about early trophoblast differentiation in the human embryo, due to lack of a proper in vitro model system. Human embryonic stem cells (hESCs) differentiate into functional trophoblast following BMP4 treatment in presence of feeder-conditioned media; however, this model has recently been challenged, based in part on comparison to mouse trophoblast-specific markers, and in part due to lack of proof for a human trophoblast progenitor population. We have previously shown that p63, a member of the p53 family of nuclear proteins, is expressed in proliferative cytotrophoblast (CTB), precursors to terminally-differentiated syncytiotrophoblast (STB) in chorionic villi and extravillous trophoblast (EVT) at the implantation site. Here, we show that BMP4-treated hESC differentiate into bona fide CTB by direct comparison to primary human placental tissues and isolated CTB through gene expression profiling. We show that, in primary CTB, p63 levels are reduced as cells differentiate into STB, and that forced expression of p63 maintains cyclin B1 and inhibits STB differentiation. We also establish that, similar to in vivo events, hESC differentiation into trophoblast is characterized by a p63$^+$/CK7$^+$ CTB stem cell state, followed by formation of functional KLF4$^+$ STB and HLA-G$^+$ EVT. Finally, we illustrate that downregulation of p63, by shRNA, inhibits differentiation of hESCs into functional trophoblast.

Most recently, we have developed defined culture conditions for differentiating hESCs into a pure population of CTB stem cells, based on expression of CK7, p63, as well as EGFR. We are in process of identifying optimal conditions for maintenance and subtype-specific differentiation of these CTB, into either EVT or STB. We have found that low oxygen enhances both induction of CTB and further differentiation into EVT, and inhibits STB differentiation.
Taken together, our results establish that hESCs are an excellent model for early human trophoblast lineage specification and differentiation, as they can be used to mimic the in vivo progression from $p63^+$ CTB stem cells to terminally-differentiated trophoblast subtypes. In the near future, we plan to use this in vitro system to model placenta-based pregnancy disorders, including preeclampsia and fetal growth restriction.

F-2343

THE ROLE OF PLACENTAL EXTRACELLULAR MATRIX IN TROPHOBLAST DIFFERENTIATION

Trishana M. Smith[1], Todd Johnson[2], Anna Wakeland[2], Matteo Moretto-Zita[2], Yingchun Li[2], Karen Christman[2], Mana M. Parast[2]
[1]UCSD/SDSU, San Diego, CA, United States, [2]UCSD, San Diego, CA, United States

Objective
Extracellular matrix is the biological scaffold which provides both structural support as well as factors which assist in cellular differentiation and tissue/organ function. Cytotrophoblast (CTB) are the epithelial stem cells in the placenta which can give rise to both villous syncytiotrophoblast (STB) and invasive extravillous trophoblast (EVT). Both primary CTB isolated from the human placenta and human pluripotent stem cell (hPSC)-derived CTB, following BMP4 treatment, quickly lose their proliferative potential and differentiate when cultured under standard conditions (on tissue culture plastic for primary CTB and Geltrex for hPSC-derived CTB). We set out to determine whether placental extracellular matrix (ECM) would be more optimal in maintaining the undifferentiated state of either primary or hPSC-derived CTB.
Study Design
ECM was prepared from minced term placental tissues following decellularization using SDS, Triton-X and DNase treatment. The resulting matrix was dehydrated by a lyophilizer and reconstituted in 0.1M acetic acid before plating. Primary CTB were isolated from term placental tissues per published protocols and cultured either on tissue culture plastic or 1 mg/ml placental ECM. For derivation of CTB from hPSCs, feeder-free H9 hESC line were plated on Geltrex and differentiated in feeder-conditioned media + 10 ng/ml BMP4 for four days, yielding >90% cytokeratin 7-positive cells; these cells were then replated on either Geltrex or 1 mg/ml placental ECM. Differentiation was monitored based on morphology, hCG production, and qPCR for lineage-specific markers.
Results
With isolated term CTB, cells plated on placental ECM mostly remained mononuclear, produced significantly tenfold more hCG, and showed significantly lower expression of CGA, CGB, and CSH-1 (all markers of differentiated STB) by qPCR. With H9-derived CTB, hCG production was equivalent on both Geltrex and 1 mg/ml placental ECM.
Conclusion
While placental ECM appears to maintain primary CTB in their undifferentiated state, hPSC-derived CTB continue to differentiate on this matrix. It is likely that other culture conditions, including the media, would have to be optimized for the latter cells in order to inhibit terminal differentiation.

F-2344

IN VITRO ASSAYS FOR ASSESSING PULRIPOTENCY OF HUMAN STEM CELLS

Orla O'Shea, Shalinee Khadun, Harsharon Kaur Bahia, Lesley Young, Lyn Healy, Glyn Stacey
NIBSC, UK Stem Cell Bank, Potters Bar, United Kingdom

The characterisation of human embryonic stem cells (hESCs) is a constantly evolving area that relies on consensus between researchers. The UK Stem cell Bank (UKSCB) produces banks of undifferentiated hESCs for worldwide dis-

tribution and needs to implement such scientific consensus in its testing regimes under strict quality control to ensure reliability of data published on cell lines.
Markers for phenotypic and genotypic characterisation have been described in detail in the literature and evolve as research develops. Pluripotency is a key characteristic of hESCs and is defined as the ability to give rise to cells from all three germ layers of the body. This capability can be assessed in vivo using the teratoma assay, or in vitro, by the directed differentiation of the cells into all three germ layers. Here, we describe and discuss in vitro methodologies employed by the UKSCB to characterise the pluripotent potential of deposited hESCs, with the objective of including these techniques in the routine testing of banked hESC lines. This information will add value to the lines by providing additional datasets on the potential of the individual lines to differentiate into cells of the three germ layers, facilitating the selection of hESC lines chosen by potential customers.
As part of this program of work, two different methods for differentiating human stem cells into endoderm, ectoderm and mesoderm were investigated using a number of stem cell lines. The first protocol induced differentiation via embryoid body (EB) formation in defined media conditions and the second method consisted of using the same media compositions on adherent cells. Different growth factors were used to induce differentiation along the three germ lineages. We will discuss data acquired using Activin a/wnt3a for the production of definitive endoderm, BMP4/Activin A/FGF2 for initiation of primitive mesoderm and Dual SMAD inhibition for neural induction. Differentiation was demonstrated using real time PCR and immunofluorescence staining to detect the presence of germ layer markers.
Each method of differentiation was assessed for the following criteria; ability to demonstrate the pluripotency potential of stem cell lines, differentiation efficiency, reproducibility, cost, robustness and transferability.
It was found that both the EB and adherent cell methods allowed the differentiation of all the embryonic stem cell lines and iPSCs used in this experiment. Using the EB method would be more suitable for routine characterisation testing at the UKSCB, as it is more robust, reliable, cost-effective and subject to less inter-operator variability.

F-2345

DISTINCT REGULATORY MECHANISMS UNDERLYING EFFICIENT GENERATION OF midbrain DOPAMINERGIC NEURONS (mDA) BY KEY TRANSCRIPTION FACTORS DURING IN VITRO DIFFERENTIATION OF ES CELLS

Sunghoi Hong
Korea University, Seoul, Korea, Republic of

Identification and functional characterization of key transcription factors have not only advanced our understanding of development and maintenance of midbrain dopamine (mDA) neurons but also greatly contributed to the successful generation of mDA neurons from stem or somatic cells. However, detailed functional mechanisms for key fate-determining transcription factors (e.g., Nurr1, Pitx3, and Lmx1a) and their interplay during mDA differentiation remain poorly defined. In this study, to further investigate and compare the functional roles of these mDA regulators, we developed a highly efficient gain-of-function system by transducing neural progenitors (NPs) derived from embryonic stem cells (ESCs) with retroviral vectors, allowing us to analyze downstream molecular and cellular effects following individual transcription factor overexpression. Nurr1, Pitx3, or Lmx1a overexpression robustly promoted the dopaminergic differentiation of ES-NP cells exposed to sonic hedgehog (SHH) and fibroblast growth factor 8 (FGF8). Interestingly, however, Nurr1 overexpression but not Pitx3 or Lmx1a, generated a significant number of non-neuronal tyrosine hydroxylase-positive cells. In line with this, Pitx3 and Lmx1a, but not Nurr1, induced mRNA expression of the Ngn2 gene, which plays critical roles in neurogenesis. We also observed that Pitx3 directly binds to its potential binding sites (SNTAATCCM) within the tyrosine hydroxylase (TH) gene, suggesting that mDA neuronal development is regulated by the direct interaction of Pitx3 with the TH gene. Taken together, our data demonstrates that key mDA regulators play overlapping as well as distinct roles for neurogenesis and neurotransmitter phenotype in generation and maintenance of mDA neurons.

F-2346

HIGHLY EFFICIENT DERIVATION OF MOTONEURONS FROM HUMAN EMBRYONIC STEM CELLS USING BAC TRANSGENESIS

Elizabeth L. Calder[1], Dimitris G. Placantonakis[2], Mark J. Tomishima[3], Viviane Tabar[4], Lorenz Studer[5]
[1]Weill Cornell Graduate School of Medical Sciences, New York, NY, United States, [2]Department of Neurosurgery, New York University, New York, NY, United States, [A]SKI Stem Cell Research Facility, [B]Department of Neurosurgery, [C]Developmental Biology Program, [3]Sloan-Kettering Institute, New York, NY, United States

Amyotrophic Lateral Sclerosis and Spinal Muscular Atrophy are characterized by degeneration of motoneurons (MNs) resulting in a loss of voluntary motor control, diminished quality of life, and death. Human pluripotent stem cells (hPSCs) represent an unlimited source for generating MNs to study MN disease pathophysiology and explore therapeutic applications of hPSCs to treat MN diseases. The advent of induced pluripotent stem cells (iPSCs) has permitted the ability to generate patient-specific MNs to measure to what extent a genetic background contributes to MN diseases. While patient-specific iPSCs may also hold great promise for drug discovery and potential cell replacement therapies, a more reliable, scalable strategy is required for the generation of large numbers of MNs.
In this study, we adapted the dual-SMAD inhibition protocol for early neural induction, originally developed in our lab, with subsequent MN patterning and differentiation. Retinoic acid and an activator of sonic signaling were used to specify neural progenitors into MNs that express markers characteristic of MN identity by both immunocytochemistry and qRT-PCR, including the transcription factors HB9, Isl1, and Nkx6.1 as well as choline acetyltransferase. Using a spinal MN specific HB9::GFP hESC reporter line, we optimized the duration and timing of patterning factors to develop a well-defined, small molecule-based protocol. This protocol produces up to 40% HB9::GFP+ cells after 2 weeks of differentiation. We plan to further elucidate the importance of signaling pathways required for MN specification that contribute to a highly efficient, streamlined protocol for MN derivation from human pluripotent cells and to establish a human-based framework for developmental studies and disease modeling.

F-2347

QUANTITATIVE ANALYSIS OF PROTEIN DYNAMICS IN DIFFERENTIATING MOTOR NEURONS

A. Ertugrul Cansizoglu[1], Tina Hempel[1], Eunju Chang[2], Lee Rubin[2], Judith Steen[3]
[1]Neurology, HMS and Boston Children's Hospital, Boston, MA, United States, [2]Harvard Stem Cell Institute, Boston, MA, United States, [3]Neurology, Boston Children's Hospital, Boston, MA, United States

Stem cell research has been the most promising development in neurodegenerative medicine. The field has tremendous potential for therapy in diseases such as Amyotropic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA) which particularly affect motor neurons. One important issue with the motor neurons has been the lack of cell specific markers to monitor differentiation. We used proteomics as a research tool to investigate the mechanisms governing neuronal development. Quantitative mass spectrometry is a powerful tool to analyze the stage-specific global protein expression profiles of cells during development. Using a global proteomics approach, we determined the abundance and relative quantities of more than 4300 proteins sampled at each developmental stage for motor neurons. The protein distribution of the stage specific cells can be grouped into multiple clusters which display characteristic patterns. Certain networks show a clear regulated behavior along differentiation process falling into the same expression cluster. Total protein analysis revealed a detailed proteomics map of the developing motor neurons. When done in the context of mutant genotypes, this will ultimately help us identify new therapeutic targets for neurodegenerative and developmental disorders such as SMA. On the other hand, total analysis of protein constitution of motor neurons will allow us to identify developmental stage specific motor neuron markers which will aid research on motor neurons in general. As genomics will give information upon the expression profiles of proteins; proteomics, in parallel, will complement the downstream regulation of the differentiating cells at the protein level.

F-2348

A UNIQUE CO-CULTURE SYSTEM OF DIFFERENTIATING MOTOR NEURONS AND SKELETAL MUSCLE MYOBLASTS

Yuka Kawabata, Mami Kobayashi, Nana Takenaka, Shigeko Torihashi

Nagoya University Graduate School of Medicine, Nagoya, Japan

Embryonic stem (ES) cells have the potential to generate all cell types in culture. Effective induction of motor neurons from ES cells will provide attractive sources for regenerative medicine including amyotrophic lateral sclerosis and spinal muscular atrophy. In the embryo, the development of motor neurons in the brain and spinal cords extend axons to their targets such as other neurons and skeletal muscle fibers .From the spinal cords, motor neurons extend their axons to skeletal muscle cells forming neuromuscular junctions and muscle contractions are achieved thereafter. On the other hand, ES cells can differentiate into motor neurons under treatment with retinoic acid and sonic hedgehog. Besides them, various factors are involved in the formation of neuromuscular junctions under the interactions between motor neurons and skeletal muscle cells, however, much has remained unclear. To clarify this, co-culture systems in which two cell types are maintained at the same time in the same chamber, are commonly used. However, the usual culture system does not mimic embryonic development *in vivo* and it is difficult to analyze the interactions between motor neurons and skeletal muscle cells precisely. We then, developed a new co-culture system using pair chambers in which motor neurons derived from mouse ES cells and C2C12 myoblasts were incubated independently. We attached the dual chamber in a 35mm dish and made two individual small chambers in the dish. First, we incubated C2C12 myoblasts in one side and differentiated them into myotubes; we then cultured neuronal precursor cells derived from mouse ES cells in the other side of the chamber. After a certain time period, walls of the pair chambers were removed and two types of cells were finally co-cultured. We analyzed the effects of skeletal muscle myoblasts and myotubes on the growth of motor neurons derived from mouse ES cells. As a result, we found that the number of survival motor neurons increased and the length of the extending axons was prolonged. The results indicate that skeletal muscle myoblasts and/or myotubes released certain growth factors to promote neuronal growth.

F-2351

GENERATION OF MATURED MOTOR NEURONS FOR USE IN DISEASE MODELING AND PREDICTIVE TOXICOLOGY

Fan Yang, Aarathi Shanmugan, Huan Tran, Diana L. Clarke, Thomas Fellner
Pluripotent Stem Cell Innovation Center, Lonza Walkersville, Inc., Walkersville, MD, United States

Human Pluripotent Stem Cells (hPSCs) offer a new approach to understanding early human development and ultimately altering disease progression. Generation of motor neurons from human induced pluripotent stem cells (hiPSCs) now makes it possible to not only model human motor neuron development, but express disease phenotypes and follow pathogenic and toxicological processes. To have a relevant population of cells for these applications, these cells must follow specific pathways of neural differentiation and express morphological and functional characteristics that exemplify functioning motor neurons *in vivo*. We have investigated changes in morphological and electrophysiological properties associated with the differentiation and maturation of motor neurons derived from hESCs and iPSCs using a previously developed differentiation protocol. By further defining the maturation conditions, we show robust formation of early neuroectodermal cells which organize into rosettes and late neuroectodermal cells that express both HB9 and Sox1. As motor neuron maturation advances, differentiating cells increase their neurite complexity and express Isl-1, choline acetyltransferase (ChAT) and SMI32. Membrane properties also change in a subset of these mature motor neurons and include the appearance of a repetitive firing response to sustained depolarizing stimuli. The temporal addition of these new maturation factors has significantly enhanced the differentiation of hESCs and hiPSCs to motor neurons and decreased the proportion of proliferating progenitor cells present in our cultures making them a more suitable model system.

F-2352

NEURONAL LINEAGE DIFFERENTIATION OF EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS WITH THE AID OF CADHERIN BASED MATRIX

Amranul Haque, **Nihad Adnan**, Ali Motazedian, Koichi Kutsuzawa, Toshihiro Akaike
Tokyo institute of Technology, Yokohama, Japan

N-cadherin is one of the most studied cell-cell adhesion molecules (CAMs) involved in neurite extension (formation of axon and dendrite), but how exactly it influences the differentiation of ES/iPS cells into neural or glial progenitors remains a mystery. To decipher the mechanism we synthetically engineered CAMs, viz., E-cadherin and N-cadherin fused to IgG Fc, abbreviated as E-cad-Fc and N-cad-Fc, respectively. Previously, we differentiated hepatic and neuronal lineage cells in monolayer from mouse embryonic stem (ES) and induced pluripotent stem (iPS) cells using these CAMs based matrices. Our current study focuses on neuronal lineage specific differentiation of ES/iPS cells on N-cadherin based matrix to reveal the mechanism of axon formation. We used 3D culture systems such as serum free embryonic body (SFEB), and hanging drop embryonic body (HDEB) techniques, using specific chemical growth factors, where the differentiated ES/iPS cells responded to immobilized N-cad-Fc chimera by extending longer neurites than controls. We have found that axon formation is dependent on Rho-GTPase pathway, rather than FGFR activation. Moreover, N-cadherin mediated homophilic interaction enhanced survivability of dissociated neurospheres, enriched neural population and eliminated undifferentiated ESCs. These data suggest that, cell adhesion molecules (CAMs) in association with substrate-associated adhesion molecules, can stimulate neurite outgrowth.

F-2353

INVESTIGATION INTO THE ABILITY OF REPROGRAMMED NEUROBLASTOMA CELLS TO DIFFERENTIATE INTO NEURAL CREST PROGENITORS

Jim Hackland, Christian Unger, David Preskey, Peter Andrews
BMS, University of Sheffield, Sheffield, United Kingdom

The neural crest is a migratory tissue that plays a role in early development contributing to a wide range of tissues throughout the body. Neuroblastoma is one of a group of tumours that are thought to arise from the neural crest as a result of defects in the development of the tissue. Using the hES cell line Shef6 we have developed a robust protocol for the differentiation of human pluripotent stem cells into putative neural crest progenitors. These cells, together with other hES and hiPS cell lines we have tested, express high levels of the surface antigens p75 and HNK-1 when subjected to this protocol. After cell sorting we showed, using qPCR, that expression of the neural crest markers SOX10 and AP2α segregate to the $p75^{High}$ population in a

reproducible manner. The pattern of expression of these factors and the neural plate border specifier PAX3 provides insights into the possible mechanisms of lineage specification of the neural crest and neurectoderm.
The generation of neural crest cells from a reprogrammed neuroblastoma cell line provides the opportunity to study *in vitro* the effect of genetic and epigenetic elements of the cancer in the tissue where it is thought to first manifest. We have found that hiPS cells derived by reprogramming neuroblastoma cells are similarly able to form putative neural crest progenitors as identified by high expression of p75. This indicates that possession of a neuroblastoma genotype does not impede the formation of $p75^{High}$ neural crest progenitors implying that the genetic defects may act at a later stage. This system provides a platform to model other neurocristopathies such as Hirschsprung's disease and melanoma allowing us to test how the neurocristopathic phenotype is modulated by genotype and epigenetics.

F-2354

NEURAL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS IN 3D CULTURES

Simona Casarosa[1], Angela Bozza[1], Emily Coates[2], Tania Incitti[1], Andrea Messina[1], Roberta Defilippis[1], John P. Fisher[2]

[1]Centre for Integrative Biology - CIBIO, University of Trento, Trento, Italy, [2]Fischell Department of Bioengineering, University of Maryland, College Park, MD, United States

Most mammalian tissues, including the nervous tissue, show very little capability for self repair following injury. For this reason, the interest in regenerative medicine approaches is increasing. Among these approaches, an interesting one is the possibility to differentiate pluripotent cells (such as embryonic stem cells, ESCs) in vitro to be used in cell replacement strategies. Some neural differentiation protocols for ESCs are based on monolayer cultures (Ying *et al.*, 2003, Chamber *et al.*, 2009). However, it is well known that stimulation during embryogenesis from the surrounding environment is important for cell differentiation, and that cell-cell or cell-extra cellular matrix (ECM) interactions influence and drive differentiating cells towards the right lineage. For this reason many differentiation protocols start from the generation of embryoid bodies (EBs), 3D aggregates of few thousands cells that mimic embryonic environment (Lee *et al*., 2000), even if EB differentiation is not easily reproducible. Biomaterials could be an interesting alternative allowing a more directed 3D differentiation of stem cells. The aim of this project is to evaluate the suitability of an alginate biomaterial to be used for 3D cultures in order to enhance differentiation of mouse (m)ESCs towards neural lineages.
Alginate was chosen as candidate biomaterial, either alone or modified with different ECM derived proteins and we tested whether encapsulation of mESCs within alginate beads could support and enhance neural differentiation with respect to 2D cultures. We chose to supplement alginate with the following proteins: 1. the adhesion protein fibronectin (fn); 2. the fn adhesion peptide RGD; 3. hyaluronic acid (HA, one of the major components of the neural ECM during development). After checking mESCs viability within beads, cells were cultured with a neural differentiation protocol (modified from *Fico et al.,* 2008). In few days cells form clusters and the majority of them is still alive at the end of the protocol. Quantitative Real Time (qRT)-PCR analyses showed that cells grown in alginate and alginate-HA differentiated show an increased differentiation toward neural lineages with respect to the 2D control and to fn and RGD modifications, with higher expression levels of the neural markers BIII-tubulin and NCAM. Immunocytochemistry analyses for BIII-tubulin confirmed these results both in alginate and alginate-HA experimental groups, which could be the best candidates in order to support and enhance mESCs neural differentiation. Immunocytochemistry also revealed that cells were able to make connections among themselves inside a cluster but not among clusters. Our data show that alginate, alone or modified, could be a suitable biomaterial to increase in vitro differentiation of pluripotent cells toward neural fates.

F-2355

A UNIFYING NEURAL DIFFERENTIATION PLATFORM TO DIRECT LINEAGE-SPECIFIC NEURONS AND GLIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Christine Y. Chen, Vi Chu
EMD Millipore, Temecula, CA, United States

Previously we reported development of a robust neural differentiation protocol that can efficiently generate greater than 90% neurons from starting pluripotent cultures. We now extend this neural differentiation platform to direct differentiation to lineage specific neural cells including mid-brain dopaminergic neurons, glutaminergic neurons, nociceptor neurons and oligodendrocytes. Starting with PAX6 and N-Cadherin positive neural rosettes obtained from the 10-day differentiation protocol, the effects of extrinsic signal modulating factors and small molecules that are known to play a role in patterning neural development were examined. Purmorphamine, an activator of the hedgehog signaling pathway and FGF8 yielded approximately 70% TH positive dopaminergic neuron. A PKA pathway activator directed neural cells to greater than 80% V-Glut positive glutaminergic neurons while the introduction of Notch and FGF receptor inhibitors facilitated significant nociceptor neuronal differentiation. Additionally, our results suggest that oligodendrocyte progenitor cells could be more efficiently obtained from the more primitive Pax-6 and N-Cadherin-positive neural rosette populations than from the later Nestin-positive neural progenitor cell stage.
In summary, we suggest that the Pax-6 and N-cadherin positive cell population may be more primed for neural cell patterning and may thus be a useful platform from which to study effects of developmental growth cues and signaling regulators on cell lineage specification.

F-2356

A GENOME-WIDE SCREEN TO INVESTIGATE THE ROLE OF GENE DOSAGE ON NEURAL DIFFERENTIATION

Fiona J. Coutinho[1], Simon Fortier[2], Guy Sauvageau[2], Peter B. Dirks[3]
[1]Molecular Genetics, University of Toronto, Toronto, ON, Canada, [2]Molecular Genetics of Stem Cells Laboratory, Institute of Research in Immunology and Cancer, Montreal, QC, Canada, [3]Developmental and Stem Cell Biology, Hospital for Sick Children, Toronto, ON, Canada

Mechanisms governing the generation of differentiated neurons from neural stem cells remain incompletely understood. In particular, the role of gene dosage on the events directing this differentiation has not been thoroughly investigated. Few studies have addressed the phenotypic contributions of gene dosage on neural differentiation, and none have investigated it on a genome-wide level. This lack of knowledge leaves a substantial gap in our understanding of the important cellular processes that occur during these stages of development. We sought to identify novel genes involved in neurogenesis in a dose-dependent manner. We performed an in vitro neural differentiation screen of a mouse embryonic stem (ES) cell haplodeletion library in which over 25% of the mouse genome is covered in variable-length overlapping and nested deletions resulting in clonal populations with varying haplodeficient ES cells. We assessed ES to neuronal differentiation in an 11-day experiment, and identified hits as clones that fail to express the neuronal marker βIIItubulin or show appropriate neuronal morphology. Of approximately 1300 clones screened, 25 were identified as screen hits. Further investigation confirmed 12 clone hits, all of which achieve the Nestin+ neural progenitor status but fail to progress to neurons. Additionally, these clones show variable ability to express endodermal and mesodermal lineage markers in embryoid body assays in vitro. Five of the hit clones overlap the same genomic region and were chosen to pursue with further investigation. The genomic region of interest spans approximately 700kb. Work is currently underway to exogenously express the genes of interest in the deletion clones to identify the ability to rescue the neuronal differentiation phenotype. Pathway analyses show an enrichment in metabolism-related functions though further investigation is required to identify a functional role in neural differentiation.

F-2357

GENERATION OF CLINICALLY RELEVANT HYPOTHALAMIC NEURONS FROM HUMAN PLURIPOTENT STEM CELLS

Florian Merkle[1], Asif Maroof[2], Takafumi Wataya[5], Yoshiki Sasai[5], Lorenz Studer[6], Kevin Eggan[3], Alexander Schier[4]

[A]Molecular and Cellular Biology; Stem Cell and Regenerative Biology, [1]Harvard Univ, Cambridge, MA, United States, [C]Stem Cell and Regenerative Biology, [D]Molecular and Cellular Biology, [1]Harvard Univ, Cambridge, MA, United States, [2]Center for Developmental Biology, RIKEN, Kobe, Japan, [3]Center for Stem Cell Biology, Sloan-Kettering Institute for Cancer Research, New York, NY, United States

The hypothalamus contains a diversity of neuronal subtypes relevant to human diseases that range from narcolepsy and obesity to hypertension and infertility. Here, we report the generation of neuropeptidergic hypothalamic neurons from human pluripotent stem cells (hPSCs). Hypothalamic neurons were generated using two complementary differentiation strategies: self-patterning and directed differentiation. The self-patterning strategy yields a broader array of cell types, while the directed differentiation strategy is more robust. More specifically, we generated neurons that expressed melanin concentrating hormone (MCH), arginine vasopressin (AVP), thyrotropin releasing hormone (TRH), hypocretin (HCRT), corticotropin releasing hormone (CRH), oxytocin (OXT), prooopiolemelanocortin (POMC), and agouti related peptide (AGRP). Stem cell-derived human hypothalamic neurons share characteristic morphological properties and gene expression patterns with their counterparts in vivo, and survive transplantation into the mouse brain. Since these cells are central regulators of important physiological and behavioral processes, they could be therapeutically harnessed to treat many human diseases.

F-2358

EFFICIENT DIFFERENTIATION OF HUMAN EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS INTO TARGETED MESODERM AND ENDODERM CELL FATES

Albert Q. Lam, Benjamin S. Freedman, Ryuji Morizane, Todd Valerius, Joseph V. Bonventre
Renal Division, Brigham and Women's Hospital, Boston, MA, United States

Human pluripotent stem cells (hPSCs) have the potential to generate the wide diversity of human cell types, but certain lineages, such as the intermediate mesoderm (IM) from which the genitourinary tract arises, have proven more challenging to derive. Here we report a highly efficient system to induce mesendoderm, mesoderm, and endoderm differentiation in human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) using simple, chemically defined monolayer culture conditions. For all differentiation experiments, hESCs or hiPSCs were plated as single cells onto Geltrex-coated wells in mTeSR1 medium and cultured for 2-3 days until they reached 50% confluence. To induce mesendoderm differentiation, cells were treated with serum-free media supplemented with the small molecule GSK-3β inhibitor CHIR99021 (CHIR). Differentiation into endodermal or mesodermal cell types was achieved by first inducing mesendoderm with CHIR, then adding specific combinations of growth factors or small molecules at precise time intervals. Treatment of hPSCs with CHIR rapidly induced BRACHYURY+MIXL1+ mesendoderm differentiation with nearly 100% efficiency in a manner which recapitulated mesendoderm formation during embryonic development. CHIR-induced mesendodermal cells could be robustly differentiated into endodermal or mesodermal lineages with the precisely-timed addition of Activin A, BMP, and FGF signals. The combination of CHIR and Activin A robustly generated SOX17+ definitive endoderm, which could subsequently be differentiated into albumin-expressing hepatocytes, hormone-producing pancreatic endocrine cells, and the precursors to lung, thyroid, and intestinal tissue. Optimization of CHIR exposure times and the addition of BMP4 and FGF2 resulted in mesoderm differentiation. Consistent with the role of BMP4 in patterning the mesoderm during development, treating CHIR-induced cells with increasing doses of BMP4 promoted the differentiation of lateral plate mesoderm and inhibited differentiation of paraxial mesoderm, as assayed by FOXF1 and TBX6 expression, respectively. FGF2 promoted the expression of markers of all three subtypes of mesoderm, demonstrating its role as a pan-mesoderm inducing factor and, importantly, strongly induced the expression of PAX2, one of the earliest markers of IM. The addition of retinoic acid to FGF2 resulted in robust differentiation of hPSCs into PAX2+LHX1+ intermediate mesoderm cells, precursors to human kidney tissue. When cultured for an additional 2 weeks in media promoting renal epithelial cell growth, these IM cells differentiated into tubular-like structures which stained positive for Kidney-specific protein (KSP) by immunocytochemistry. Our findings establish a system whereby hPSCs can be flexibly and robustly differentiated into various endodermal or mesodermal lineages, including kidney progenitors, for regenerative medicine.

F-2361

TRF2 FUNCTIONS AS A REGULATOR OF HUMAN EMBRYONIC STEM CELL NEURAL DIFFERENTIATION

Patrick Ovando-Roche, Wei Cui
Surgery & Cancer, Imperial College London, London, United Kingdom

Telomere shelterin proteins have an essential role in the protection, maintenance and regulation of telomere integrity, consequently contributing to genomic stability and normal function in cells. Telomere binding protein TRF2 is an essential component of shelterin for telomere protection. Depletion of TRF2 leads to deprotection of the telomere ends and initiation of the DNA damage response leading to telomere attrition and cell senescence. However, compelling evidence shows that not all cells respond to TRF2 deletion in the same way. In neuroblastoma and embryonal carcinoma stem cells inhibition of TRF2 promotes cell differentiation towards the neural lineage, suggesting an extra-telomeric role of TRF2 in neural differentiation. In an attempt to explore the role of telomere binding proteins TRF1 and TRF2 in human embryonic stem cell (hESCs) differentiation, we have found that H1 and H7 hESCs have strikingly low levels of TRF2 protein and that these are dramatically upregulated upon differentiation to neural progenitor cells (NPCs) and then downregulated back to low levels when NPCs are terminally differentiated into neurons or glia. This differential expression of TRF2 was not observed when hESCs were differentiated to other lineages, indicating that TRF2 may indeed play a role in neural differentiation, which is distinct from its well known role in chromosome end protection. To further address TRF2's role in neural differentiation, we generated TRF2 overexpressing/knockdown hESCs and NPCs using lentiviruses and characterised these cell lines using several different methods including RT-PCR, telomere length assays, FISH, immunocytochemistry and western blotting. While overexpression of TRF2 in hESCs hindered their self-renewal and induced NPCs differentiation, TRF2 knockdown in NPCs dramatically affected their capacity to terminally differentiate into mature neurons or glia. We demonstrated that the increase or decrease of TRF2 protein levels positively regulated the protein levels of the REST splice variant REST4 but had no significant effect on REST. Furthermore, we showed by co-immunoprecipitation assays that, TRF2 interacts with REST4 to form a protein complex while interaction with REST is not observed. These data suggests that TRF2 may regulate neural differentiation of hESCs by interacting and stabilising REST4 which has reduced repression potential and can outcompete binding of REST to its neural target genes, allowing the derepression of these genes and thus poising the cells to become NPCs.

F-2362

IN SILICO MAPPING OF METABOLOME REVEALS UNIQUE METABOLOMIC FINGERPRINTS IN HESC-DERIVED GLUTAMATERGIC NEURONS

Soumya Pati, Li-hua Ma, Xu Han, Cemal Karakas, Mirjana Maletic-Savatic
Pediatrics, Baylor College of Medicine, Houston, TX, United States

Metabolomics has emerged as new tool to study metabolic changes associated with the cell states in a variety of diseases. To decipher distinct metabolic signatures associated with stage specific development of human embryonic stem cell (hESC)-derived glutamatergic neurons, we have determined their metabolome fingerprints. First, the purity of the glutamatergic neurons was ascertained by fluorescent activated cell sorting using vGlut1 as a specific marker of these neurons. Metabolomic data were acquired by high field NMR (800MHz Bruker) and analyzed by Chenomx. We found 93 metabolites comprising of amino acids, nucleotides, carbohydrates, Krebs cycle products, lipids and derivatives and glycerophospholipids. Out of these 93 metabolites, 24 were significantly different between hESCs and glutamatergic neurons. Most metabolites were highly expressed in hESC, apart from myo-inositol and sn-glycero-3-phosphocholine. Identification of metabolic pathways for candidate metabolites was performed by in silico mapping to existing databases. Integrated mapping demonstrated certain metabolic pathways predominantly existing in hESCs, such as those related to gamma-aminobutyric acid, glycine, serine, threonine, and glucose metabolism. In glutamatergic neurons the most prominent pathways involved glycerolipid and inositol-related metabolites. Overall, in silico mapping of metabolic profiles of hESC-derived neurons has identified unique

sets of metabolic fingerprints involved in neuronal fate commitment of hESCs and provided new knowledge on the metabolic biomarkers of neuronal development.

F-2363

PROSTAGLANDIN E2 REGULATES CELL FATE DECISIONS AND ORGAN GROWTH DURING LIVER AND PANCREAS DEVELOPMENT

Sahar Nissim[1], Richard Sherwood[2], Julia Wucherpfennig[2], Diane Saunders[2], Gregory Frechette[3], James Harris[3], Claire Cutting[2], Trista E. North[4], Wolfram Goessling[5]

[1]Brigham and Women's Hospital, Division of Gastroenterology, Boston, MA, United States, [2]Brigham and Women's Hospital, Division of Genetics, Boston, MA, United States, [3]Beth Israel Deaconess Medical Center, Department of Pathology, Boston, MA, United States, [4]Beth Israel Deaconess Medical Center, Department of Pathology; Harvard Stem Cell Institute, Boston, MA, United States, [5]Brigham and Women's Hospital, Division of Gastroenterology and Division of Genetics; Harvard Stem Cell Institute, Boston, MA, United States

The liver and pancreas arise from common endodermal progenitors. How these distinct cell fates are specified is poorly understood. We uncover PGE2 as a novel regulator of endodermal fate specification, revealing a previously unrecognized developmental role for this pathway. Modulating PGE2 activity has opposing effects on liver versus pancreas specification in zebrafish embryos. We find a similar effect of PGE2 on bipotential endoderm progenitors derived from mouse embryonic stem cells, demonstrating mammalian conservation for this role. Consistent with a role in liver versus pancreas cell fate decisions, the PGE2 receptor ep2a and synthetic enzyme cox2a are patterned such that cells closest to the source of PGE2 acquire a liver fate whereas more distant cells acquire a pancreas fate. At later stages, PGE2 promotes the outgrowth of the nascent liver and pancreas. Genetic knockdown and pharmacologic blockade of the PGE2 synthetic pathway and receptors ptger2 and 4 abrogates liver and pancreas development, providing the first in vivo demonstration that PGE2 activity is necessary for normal embryonic development of these endodermal derivatives. Conversely, inhibiting the PGE2 metabolizing enzyme prostaglandin dehydrogenase results in increased embryonic liver and pancreas size, corroborating a role for endogenous PGE2 in outgrowth of these organs. We extend these findings to adult zebrafish and show that PGE2 modulates liver regeneration and tumor formation. This work provides the first in vivo evidence that PGE2 regulates cell fate decisions and outgrowth of the embryonic liver and pancreas. This insight may lead to the development of novel therapeutics for liver and pancreas regeneration and cancer in the adult.

F-2364

MODULATION OF EMBRYONIC STEM CELL-DERIVED CHONDROCYTE AND OSTEOBLAST DIFFERENTIATION BY THE RHO/ROCK GTPASE PATHWAY

Dalea M. Bukhary[1], April Craft[3], Fraser McDonald[2], Gordon Keller[3], Agi E. Grigoriadis[1]

[A]Dept Craniofacial Development and Stem Cell Biology, [B]Dept Orthodontics, [1]Kings College London, London, United Kingdom, [2]McEwen Centre for Regenerative Medicine, University Health Network, Toronto, ON, Canada

In vitro differentiation of Embryonic Stem Cells (ESCs) and induced Pluripotent Stem Cells (iPSCs) is now a well-established approach for modeling embryonic development through germ layer induction and for understanding the molecular mechanisms underlying the commitment and and expansion of specific lineages. We have used pluripotent stem cell technology and have developed a novel mESC differentiation system for investigating the mechanisms of chondrocyte and osteoblast lineage commitment and differentiation. This step-wise, serum-free protocol uses specific recombinant factors to investigate (a) the mechanisms of pluripotent stem cell commitment to mesoderm and bone/cartilage cell lineages, and (b) the role of Rho GTPase signaling in ESC-derived chondrocyte/osteoblast differentiation. Activation of the Nodal/Activin and canonical Wnt pathways together with inhibition of BMP signaling (Noggin) directed ESCs to form a primitive streak-like population expressing Brachyury, which was further

enriched to mesodermal subpopulations expressing PDGFRa, lateral plate and paraxial mesoderm markers. These populations exhibited reduced hematopoietic/osteoclastic and cardiac potentials but differentiated efficiently in monolayer culture to chondrocyte and osteoblast lineages. Inhibition of Rho/ROCK signaling using the ROCK inhibitor, Y-27632, at different stages of mesoderm enrichment and differentiation phases modulated expression of PDGFRa and the paraxial mesoderm markers TCF15 and TBX18, resulting in a 2 to 3-fold increase in chondrogenesis and osteogenesis as measured by cartilage and bone nodule formation, respectively. This was confirmed by qPCR analysis of chondrocyte (Sox9, Coll2)- and osteoblast (Runx2, ALP, BSP)-specific genes, as well as by Alcian blue staining and Coll2 antibody staining of differentiated chondrocyte monolayers. Preliminary data also suggest that differential exposure to bFGF and BMP4 together with stage-specific addition of Y-27632 enhanced differentiation and/or expansion of hypertrophic chondrocytes and mineralizing osteoblasts. Finally, renal capsule grafting studies showed that the ESC-derived mesodermal populations gave rise to both cartilage and bone in vivo, mimicking endochondral ossification. The ESC model system provides defined, manipulatable and expandable chondro-osteoprogenitor populations that will provide insights into the molecular basis of bone/cartilage development and disease, as well as for generating specific populations for bone and cartilage tissue repair and replacement.

F-2365

EXOGENOUS SUPPLEMENTATION WITH ACTIVIN A BOOSTS GERM CELL DIRECTED DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

Galbha Duggal[1], Bjorn Heindryckx[1], Margot Van der Jeught[1], Sylvie Lierman[1], Tom Deroo[1], Dieter Deforce[2], Susana Chuva de Sousa Lopes[3], Petra De Sutter[1]

[1]Department for Reproductive Medicine, Ghent University Hospital, Ghent, Belgium, [2]Laboratory for Pharmaceutical Biotechnology, Ghent University, Ghent, Belgium, [3]Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands

Mouse epiblast stem cells are poised towards germ cell differentiation and are derived in the presence of Activin A (ActA). ActA has also been shown to contribute to the induction of meiosis in postnatal ovaries of mice by suppressing the retinoic acid inhibitor CYP26B1. These properties of ActA urged us to investigate whether supplementation with ActA can increase germ cell gene expression during differentiation of human embryonic stem cells (hESC) towards germ cell lineage.
For our experiments, two hESC lines were differentiated as embryoid bodies (EBs) for 7 days in bFGF-free medium. We compared 4 conditions by supplementing the medium with SB431542 (SB, inhibitor of Activin signaling, 10µM), ActA (20ng/ml), BMP4 (50ng/ml), and ActA + BMP4. Non-supplemented medium served as a control.
Following differentiation, EBs were analysed by immunocytochemistry for the pluripotency marker Oct4 and the pre-meiotic germ cell marker Vasa. EBs were also analysed by qRT-PCR for the early primordial germ cell (PGC) markers Stella and cKIT, for the pre-meiotic germ cell markers Dazl and Vasa, and for the pluripotency markers Oct4 and Nanog. Two biological replicates of each hESC line were analysed.
In both hESC lines, qRT-PCR indicated a significantly higher expression for the early PGC markers Stella and cKIT in the presence of ActA ($p<0.001$). In the presence of BMP4, Stella and cKIT expression was lower, while the late PGC markers Dazl and Vasa were significantly upregulated compared to control, SB and ActA conditions ($p<0.05$). Remarkably, ActA+ BMP4 combination further increased Dazl and Vasa expression significantly compared to all conditions (Dazl: versus control, SB, ActA, BMP4; $p<0.005$) (Vasa: versus control, SB, ActA; $p<0.001$, versus BMP4; $p<0.05$). Both Oct4 and Nanog were highly expressed in the presence of ActA, downregulated in BMP4-only conditions and upregulated again when ActA and BMP4 were combined (versus control and SB; $p<0.001$).
Similar observations were seen at the protein level. In the presence of ActA, few Vasa positive cells were observed along with Oct4 positive clusters occurring in different areas of the EBs. Vasa was rarely seen to co-localize in these Oct4 clusters. In the presence of BMP4, several Vasa positive cells were observed and almost no Oct4 positive cells existed. Interestingly, in the presence of ActA+BMP4, abundant Vasa expression was seen, concurrent with few Oct4 positive cells/clusters. In EBs cultured in medium without supplementation or with SB, Vasa expression was undetectable and Oct4 was completely absent.

In conclusion, ActA appears to prime hESCs towards the germ cell lineage, as seen by the high expression of the early PGC markers Stella and cKIT. However, low expression of the late PGC genes Dazl and Vasa shows that additional factors are required to further develop hESCs into late germ cell precursors. Indeed, the combination of ActA with BMP4 strongly increases germ cell differentiation potential of hESCs based on the high expression of Dazl and Vasa. Hence, although BMP4 is widely known to direct germ cell differentiation in hESCs and is able to produce increased Dazl and Vasa expression by itself, the combination of ActA with BMP4 seems to provide an additional boost to hESCs to develop into post-migratory germ cells. Further studies will be conducted to validate the mechanistic and functional role of ActA during germ cell differentiation of hESCs and to study its involvement in conferring meiotic competency to *in vitro*-derived germ cells.

F-2366

BMP SIGNALING REGULATES THE DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS INTO LUNG EPITHELIAL CELL LINEAGES

Naoto Ninomiya[1], Tatsuo Michiue[1], Makoto Asashima[2], Akira Kurisaki[2]
[1]University of Tokyo, Tokyo, Japan, [2]National Institute of Advanced Industrial Science and Technology (AIST), Ibaraki, Japan

Somatic stem/progenitor cells are known to be present in most adult tissues. However, those in the lung have limited abilities for tissue regeneration after serious damage as a result of chronic disease. Therefore, regenerative medicine using exogenous stem cells has been suggested for the treatment of progressive lung diseases such as chronic obstructive pulmonary disease and pulmonary fibrosis. Embryonic stem (ES) cells and induced pluripotent stem cells, with their potent differentiation abilities, are promising sources for the generation of various tissue cells. In this study, we investigated the effects of various differentiation-inducing growth factors on the differentiation of lung cells from ES cells in vitro. Several factors, including activin, nodal, and noggin, significantly promoted the induction of Nkx2.1-positive lung progenitor cells when cells were cultured as embryoid bodies. Bone morphogenetic protein (BMP) 4 signaling controls the lineage commitment of lung cells along the proximal-distal axis. BMP4 promotes the induction of distal cell lineages of alveolar bud, such as Clara cells and mucus-producing goblet cells. These results suggest that several developmentally essential factors, including nodal/activin and BMP signaling, are important in the control of the differentiation of lung epithelial cells from mouse ES cells in vitro.

F-2367

EXTERNAL FORCES APPLIED ON EMBRYONIC STEM CELLS IN 3D CAN DIRECT EARLY DIFFERENTIATION TOWARD A SPECIFIC GERM LAYER.

Dekel Dado-Rosenfeld, **Shulamit Levenberg**
Biomedical Engineering, Technion, Haifa, Israel

Controlling embryonic stem (ES) cell proliferation and differentiation to form complex viable three-dimensional (3D) tissues is challenging due to their pluripotency and their potential therapeutic implications. We have previously shown that polymer scaffolds which serve as mechanical and biological supports for cell growth and func-

tionality can promote proliferation, differentiation and organization of ESCs into 3D structures. In addition, we recently demonstrated that scaffold elasticity can influence differentiation of hESCs. Herein, we investigated whether external forces applied on ESCs grown on 3D matrices can mimic processes in embryogenesis and direct ESC early differentiation toward specific germ layer. For this, we applied mechanical manipulations on collagen seeded constructs using an advanced computer controlled bioreactor.
Our results show that external signals mediated to the cells through the scaffold can induce their differentiation with a different response for each of the germ layers. Preference to the mesodermal direction was observed under oscillatory forces supported by upregulation of representative genes of the mesoderm layer. Downregulation in representative genes of the ectoderm and endoderm layers was observed. Additionally, under the effect of blebbistatin, an inhibitor of myosin II, the highest downregulation in brachyury expression was observed, demonstrating the high impact of external forces on ES cell differentiation into the mesodermal direction.
Altogether, our results show that external forces applied on embryonic stem cells in 3D through their matrix can direct their early differentiation toward a specific germ layer. Controlling the differentiation through mechanical manipulation of the microenvironment can advance our understanding of developmental mechanisms and shed light on the involvement of forces in embryogenesis.

F-2368

EFFECT OF OXYGEN TENSION ON THE PULMONARY DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS.

Elena Garreta[1], Esther Melo[1], Daniel Navajas[2], Ramon Farré[1]
[1]Biophysics and Bioengineering, University of Barcelona - IDIBAPS - CIBERES, Barcelona, Spain, [2]Biophysics and Bioengineering, University of Barcelona - IBEC - CIBERES, Barcelona, Spain

The local oxygen tension is an important parameter of the stem cell microenvironment. Embryonic stem cells (ESC) live at low oxygen concentrations in vivo from the implantation stage through fetal development. Hence, the normal physiological environment of ESC is one of relative hypoxia compared with traditional in vitro culture conditions. In addition, during embryonic development, the formation of the different tissues and organs is heavily influenced by oxygen gradients. Therefore, oxygen tension is potentially a key variable that could be used to improve the controlled differentiation of ESC into a desired cell lineage in vitro. The aim of the present work is to study the effect of oxygen tension on the generation of primordial lung progenitors from mouse ESC. We have employed a stepwise differentiation protocol with appropriate growth factor stimulation to induce the generation of definitive endoderm cells competent to undergo differentiation into Nkx2.1+ endodermal lung progenitors. Differentiating cell cultures were maintained at 5% or 20% oxygen concentration during the whole process. Differentiation process was analyzed at days 0, 6 and 12 of the protocol by real time RT-PCR and immunofluorescence. At day 12, mRNA expression of the endoderm transcription factors Foxa2 and Sox17 was significantly higher at 5% O2 than 20% O2. The dual expression of these transcription factors at the protein level was analyzed by immunofluorescence staining, confirming the presence of definitive endoderm cells. In addition, at day 12 of the protocol, Nkx2.1 gene expression, the earliest gene marker of lung endoderm, was significantly higher at 5% O2 than 20% O2. Immunofluorescence staining revealed the presence of cells co-expressing Foxa2 and Nkx2.1, but negative for both Pax8 (a gene expressed in thyroid endoderm) and Tuj1 (ventral forebrain), confirming the generation of lung endoderm. In conclusion, the present data strongly suggest that 5% oxygen tension facilitates the differentiation of mouse ESC into endodermal lung progenitors.

F-2371

TRANSGENIC EXPRESSION OF GLIAL-DERIVED NEUROTROPHIC FACTOR (GDNF) PROMOTES DIFERENTIATION AND SURVIVAL OF MOUSE EMBRYONIC STEM CELL-DERIVED DOPAMINERGIC NEURONS

Daniel E. Cortés Pérez, Iván Velasco
neurodesarrollo y fisiologia, Instituto de Fisiología Celular, UNAM, Mexico city, Mexico

Embryonic stem cells (ESCs) are pluripotent and thus able to differentiate into any tissue of the three embryonic layers. Currently, there are several differentiation protocols to produce dopamine (DA) neurons from ESCs, although with low yields. It is well known that GDNF confers neuroprotection to DA cells. Exogenously added GDNF also promotes DA differentiation, but the specific phenotype of these DA neurons has not been characterized. In the present work, we engineered mouse ESCs to constitutively express GDNF in order to investigate if this trophic factor favours DA specification and terminal differentiation. We hypothesized that autocrine secretion of GDNF would allow a more robust dopaminergic differentiation and also increase DA neuron survival against a noxious stimulus. We constructed lentiviral vectors in which elongation factor 1α (EF-1α) drives the expression of human GDNF (hGDNF), and this transgenic construct is coupled to a puromycin resistance cassette. Control and hGDNF viruses were produced and ESCs were transduced and selected by antibiotic resistance. Several clones that contained the transgene were tested for markers associated to pluripotency, which were maintained in hGDNF-containing cells. Clones that expressed hGDNF mRNA, produced and secreted this protein were selected for further studies by RT-PCR, western blot and ELISA, respectively. We performed DA neuron differentiation of control and hGDNF ESCs by a five-stage protocol: control cells produced 22% of Tyrosine Hydroxylase (TH)-positive neurons, whereas this proportion was significantly increased to 44% in hGDNF cells. We studied transcriptional factors key for the proper commitment and differentiation of midbrain DA neurons. Lmx1a was significantly increased from 15% to 26%, and FoxA2 went up from 13% to 27% in hGDNF transgenic cells, when compared to control ESCs. A specific marker of A9 (*substantia nigra pars compacta)* midbrain neurons is the potassium channel Girk2, which was present in 12% of neurons in control cells, and increased to 21% in GDNF ESCs.
We next challenged ESCs-derived DA neurons with the neurotoxin 6-hydroxydopamine and quantified the number of surviving TH+ neurons 24 hours later. In control ESCs, the surviving NDA were practically inexistent but in hGDNF ESCs, survival of 50% of DA neurons was observed. This protective effect was present in both young (TuJ1+) and mature (MAP2+) neurons. We conclude that this autocrine system which expresses GDNF increased dopaminergic differentiation towards a midbrain, and specifically A9 phenotype, the same cells that are lost in Parkinson disease. Self-secreted GDNF also protects differentiated DA neurons from a widely used dopaminergic neurotoxin *in vitro.* The secretion of transgenic hGDNF could improve the survival of transplanted ESCs-derived DA neurons in an animal model of Parkinson disease.

F-2372

PLEIOTROPIC EFFECTS OF BMP4 AND TGF-β SIGNALING INHIBITION ON DEFINITIVE ENDODERM DERIVED FROM MOUSE EMBRYONIC STEM CELLS

Laertis Ikonomou[1], Keri Dame[2], Juliana Barrios[2], Darrell N. Kotton[1]

[A]Pulmonary Center and Center for Regenerative Medicine, [1]Boston University School of Medicine, Boston, MA, United States

Using an Nkx2-1-GFP mouse knock-in embryonic stem (ES) cell line, we have shown that 24-hour treatment of mouse ES cell-derived definitive endoderm (DE) with noggin for BMP4 inhibition and SB431542 for dual inhibition of TGF-β and activin signaling (NS treatment) followed by a 8-to 9-day incubation with ventralizing factors such as FGF2 and BMP4 leads to the derivation of Nkx2-1-GFP$^+$ lung/thyroid progenitors. Several other research groups have demonstrated that the NS treatment is necessary and sufficient for the derivation of anterior foregut endoderm (AFE) lineages from human and mouse ES and induced pluripotent stem (iPS) cells. To further characterize the changes in DE entailed by NS treatment we used the mouse triple knock-in ES cell line GFP-T/hCD4-Foxa2/hCD25-Foxa3. Since Foxa2 is a pan-endodermal marker and Foxa3 is expressed posteriorly, AFE is enriched in Foxa2$^+$Foxa3$^-$ cells and posterior foregut endoderm in Foxa2$^+$Foxa3$^+$ cells allowing for finer resolution studies of anterior-posterior patterning. Activin stimulation for 48-72 h was followed by a 24-h treatment with either NS or activin. Sorted Foxa2$^+$Foxa3$^-$, Foxa2$^+$Foxa3$^+$ and Foxa2$^-$Foxa3$^-$ (non-endodermal) populations were characterized immediately after sorting or after 8-day incubation in ventralizing media.

Treatment with activin alone resulted in highly efficient DE induction (90-95% $Foxa2^+$ cells) and the spontaneous posterior patterning of the resulting DE (15-35% $Foxa2^+Foxa3^+$ cells). The $Foxa2^+Foxa3^-$ fraction contained also notochordal ($Noto^+$) and ciliated ($Foxj1^+$) lineages that did not persist upon reseeding. On the other hand, there was enrichment of posterior markers such as Pdx1 and Hex1 in the $Foxa2^+Foxa3^+$ population; the latter underwent robust liver differentiation in ventralizing media as indicated by high expression levels of Afp and Alb.
The 24-h NS treatment resulted in inhibition of posterior patterning as shown by a 75-90% decrease of the $Foxa2^+Foxa3^+$ population and reduced Foxa3 levels with the concomitant induction of several anterior markers and the emergence of a non-endodermal $Foxa2^-Foxa3^-$ population. Shh was highly induced in $Foxa2^+$ cells and Tbx1 in the $Foxa2^-Foxa3^-$ population. Posterior foregut differentiation was impaired as indicated by reduced levels of liver markers in the ventralized $Foxa2^+Foxa3^+$ population. Finally, $Nkx2\text{-}1^+$ expression was higher in NS-treated cells after ventralization and it was only seen in the $Foxa2^+$ (endodermal) populations.
Our results demonstrate that NS treatment of mouse ES cell-derived DE has pleiotropic effects, rendering endodermal cells competent to respond to lung/thyroid specification cues and less competent to undergo liver differentiation. Future studies will focus on non-endodermal lineages induced by NS treatment and their potential role in in vitro lung/thyroid specification of AFE.

F-2373

DISSECTING THE ROLE OF ENDOGLIN DURING MESODERM COMMITMENT BY USING DIFFERENTIATING MOUSE EMBRYONIC STEM CELLS

June Baik
University of Minnesota, Minneapolis, MN, United States

Endoglin (Eng) is an ancillary receptor that interacts with several members of the transforming growth factor-beta (TGF-β) superfamily. During mouse embryogenesis, endoglin is expressed in the extra-embryonic mesoderm and in the heart-forming region of 7.5 days post-coitum (dpc) embryos. Eng-deficient ($Eng^{-/-}$) mouse embryos show impaired primitive hematopoiesis and cardiovascular defects, which lead to embryonic lethality approximately at 10.5 dpc. Using a doxycycline (Dox) inducible conditional expression system we have observed that overexpression of Eng during embryoid body (EB) development results in the enhanced frequency of hemangioblast and primitive erythroid (EryP) precursors, while inhibiting cardiac differentiation as evidenced by the down-regulation of the cardiac genes Nkx2.5 and Tbx5, and subsequent decreased expression of the cardiac Troponin I (cTnI). These findings demonstrate that increased hematopoiesis by Eng induction happens at the expense of the cardiac lineage. Interestingly the effect of Eng overexpression in cardiac differentiation is stage specific, suggesting that endoglin may control cardiac differentiation at an early stage. To understand the molecular mechanism behind Eng gain-of-function at this early stage, we have performed transcriptional profiling analyses of non-induced versus Eng-induced EBs. Among the up-regulated genes using this approach we focused our attention on Dishevelled 1 (Dvl1). This protein is a cytoplasmic effector of Wnt signaling, and our finding suggests that Wnt activation may be involved in Eng-mediated cardiac repression. To investigate this idea, we tested the effect of a canonical Wnt signaling inhibitor, IWR-1, in Eng-induced EB cultures. In agreement with our hypothesis the cardiac repression observed following Eng overexpression is abrogated by inhibition of the canonical Wnt signaling pathway. Interestingly we also observed that Wnt inhibition negatively affects the ability of Eng to induce hematopoiesis, indicating that the function of Eng at this early stage of development may require active Wnt signaling. Importantly, by using the BMP signaling inhibitor Dorsomorphin, we have also demonstrated that the positive effect of Eng in hematopoiesis occurs via BMP signaling, as evidenced by the decreased frequency of primitive erythrocyte (EryP) colonies as well as reduced levels of SMAD1/5/8 phosphorylation in the presence of dorsomorphin in Eng-induced cultures. Considering that the modulation of Wnt activity at this stage establishes competence to form either heart or blood in response to the BMP signals, our findings suggest that Eng may promote the commitment of the early mesodermal progenitors to the hematopoietic lineage at the expense of the cardiac lineage via modulation of BMP and Wnt signaling. These studies will provide a novel insight on the molecular mechanism controlling lineage specification.

F-2374

SIMPLE AND HIGHLY EFFICIENT METHOD TO DIFFERENTIATE ENDOTHELIAL CELLS FROM PRIMATE PLURIPOTENT STEM CELLS MAINTAINED IN A SINGLE-CELL AND FEEDER-FREE CULTURE

Rie Tatsumi[1], Yutaka Suzuki[2], Takashi Ono[2], Yosuke Kato[2], Risako Fujita[2], Tomoko Yamashita[2], Toshihiro Araki[2], Naoya Sato[2], Masami Niwa[1]

[1]Department of Pharmacology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan, [2]Discovery Screening Center, Mitsubishi Tanabe Pharma Corporation, Yokohama, Japan

Pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), proliferate indefinitely and differentiate into all cells of the body, which means their in vitro differentiation provides opportunities for obtaining a wide variety of cell types.Endothelial cells derived from primate PSCs hold much promise as a valuable tool for basic research, regenerative medicine and drug discovery. In order to realize the applications, robust and efficient techniques of PSC differentiation to the desired cells are required as well as reliable culture systems to support high-quality PSC maintenance.At the last meeting, we reported an original single-cell and feeder-free (SFF) culture system with a chemically defined MT-fCFA medium containing bFGF and Activin A, by which primate PSCs actively grow and maintain their undifferentiated state for long time by passaging with trypsin treatment. By using this culture system, primate PSCs can serve as a practical cell source with several advantages; their robust and easy maintenance as well as easy and efficient use in various experiments such as cryopreservation and gene manipulation.Here we present a new differentiation method of primate PSCs to endothelial cells (ECs). This method has been developed initially using monkey ESCs cultured in the SFF condition, and consists of 2-steps; the floating culture step for treating PSCs with glycogen synthase kinase-3β (GSK-3β) inhibitor to promote mesoderm induction via activation of Wnt signaling, and the following adherent culture step in which the PSC-derived ECs are generated and expanded in the presence of vascular endothelial growth factor (VEGF).Briefly, in our experiments, cynomolgus monkey ESC lines, $CMK6_{SFF}$ and CMK970, were treated with GSK-3β inhibitor (BIO and CHIR99021) for 3 days in floating culture in the absence of bFGF and Activin A, and then seeded on type-I collagen-coated dish with VEGF-supplemented medium. After 10-15 days, an EC population was induced from both of the ESC lines at higher efficiency; Flow cytometry analyses revealed 1) the mesodermal precursor marker VEGFR2 (VEGF receptor 2)-expressing cells were generated with approximately 30-40% efficiency, and 2) most of the VEGFR2(+) cells co-expressed the endothelial markers, PECAM-1 (platelet endothelial cell adhesion molecule-1) and VE-cadherin (vascular endothelial cadherin) on their cell-surface. In addition, the isolated VEGFR2(+) PECAM-1(+) VE-cadherin(+) cells exhibited cobble stone-like morphology and endothelial features such as capillary tube-formation and acetylated LDL uptake; indicating these monkey ESC-derived cells are functional ECs. Furthermore, we confirmed this method could be also applied for human iPSCs without so much difference in efficiency of EC differentiation.These results not only show a simple and highly efficient technique of EC derivation from primate PSCs, but are meaningful to demonstrate their mesodermal differentiation potential. Although many approaches for EC differentiation have been reported to date, our method is significant since it has been developed by using the primate PSCs that possess the advantages applicable to industrial and clinical use.Thus, we propose the EC differentiation method described here contribute as a practical way to produce primate ECs available for basic research and medical application.

F-2375

SHEAR STRESS REGULATES ENDOTHELIAL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS (MESCS) VIA THE MECHANOTRANSDUCER TRPV4

Nicole M. Jones[1], Peter Toth[2], Yanmin Zhang[1], Asrar Malik[1], Jalees Rehman[1]

[A]Department of Pharmacology, [B]Research Resources Center, [1]Univ of Illinois at Chicago, Chicago, IL, United States

Introduction: Pluripotent stem cells such as mouse embryonic stem cells (mESCs) can differentiate into vascular endothelial cells. Most differentiation experiments are conducted under static conditions, however, vascular cells in vivo are usually exposed to continuous blood flow and shear stress. Little is known about how shear stress and

resulting mechanotransduction signals can impact the of differentiation of pluripotent stem cells to endothelial cells. One possible candidate is TRPV4, the only mechanotransducer of the TRPV family expressed in fully mature endothelial cells in both human and mouse. We thus studied the TRPV4 in mESCs during differentiation into endothelial cells.

Methods and Results: Dissociated mESCs were cultured onto collagen IV in differentiation medium containing vascular endothelial growth factor (VEGF), bone morphogenic protein (BMP4) and basic fibroblast growth factor (bFGF) under static conditions. Samples were harvested at 0, 2, 4, 6, and 7 days for TRPV4 expression. TRPV4 transcript levels are present in undifferentiated cells. However, with mESC colony dissociation, mRNA levels are decreased at day 2 of differentiation. There is a steady increase of TRPV4 expression from day 2 of endothelial differentiation to day 7. Western blot analysis also reveals TRPV4 protein levels are present in undifferentiated mESCs. We also exposed differentiating mESCs to varying levels of shear stress using a Streamer Gold^{TM} device, driven by a computer-controlled peristaltic pump and continuously exposed to laminar shear stress ranging from 1 dyn/cm^2 (venous level) to 5-15 dyn/cm^2 (arterial levels) over a 24 hour period and compared with cells under static conditions.

Discussion: We report the upregulation of the mechanotransducer TRPV4 in mouse ESCs undergoing endothelial differentiation. Our preliminary data also suggest that arterial and venous shear stress affects the arteriovenous specification of differentiating mESCs. These findings point towards the importance of mechanical signals in vascular differentiation of pluripotent stem cells.

F-2376

GENERATION OF NOVEL CULTURE METHODS FOR THE DIFFERENTIATION OF MAMMALIAN PLURIPOTENT STEM CELLS INTO RETINAL NEURONS.

Tania Incitti, Andrea Messina, Angela Bozza, Roberta De Filippis, Yuri Bozzi, Simona Casarosa
CIBIO, Centre for Integrative Biology, University of Trento, Mattarello (TN), Italy

Degenerative diseases of the retina cause irreversible blindness and no cure is presently available to treat patients affected by retinal dystrophies. These diseases are all characterized by degeneration of photoreceptors and/or cells of the retinal pigmented epithelium. Among the possible therapeutic approaches, strong interest is raised by cell replacement protocols based on manipulation of stem cells or photoreceptor progenitors. We have recent evidence showing that the overexpression of high doses of Noggin, a neural inducer molecule and an inhibitor of BMPs, enables Xenopus animal cap cells to differentiate into retinal neurons in vitro and to rescue a complete and functional eye in embryos lacking one of the eye primordia (Lan et al., 2009). Due to these successful results, we decided to verify whether high doses of Noggin could elicit a similar retinal-inducing activity also in mammalian stem cells. Three lines of mouse embryonic stem cells (ESC) were cultured and differentiated both in suspension and in adhesion and treated with Noggin alone or in combination with a cocktail of growth factors acting on other signalling cascades, including Shh and Wnt. In particular, Rx1-GFP KI cells (gift of Prof. Y. Sasai, Wataya et al., 2008) were a useful tool to monitor the appearance of retinal precursors (GFP positive). Unlike recently published protocols (Eiraku et al., 2011, West et al., 2012), no extracellular matrix derivatives were added to the culture plates and, due to the abundant GFP expression, no FACS analyses were needed. Preliminary results indicated that retinal precursors are obtained when mESCs are cultured in suspension and Noggin is administered in combination with other secreted molecules. Studies are now ongoing to improve the final steps of differentiation and to verify the feasibility of the protocol for the differentiation of newly generated mouse induced pluripotent stem cells. The elucidation of the pathway through which Noggin and other factors elicit retinal fates will be a first step toward the setup of improved protocols for the differentiation of human stem cells toward retinal neurons.

F-2377

ZRF1 DRIVES EMBRYONIC STEM CELL (ESC) DIFFERENTIATION INTO NEURAL PROGENITORS AND MAINTAINS THEIR IDENTITY

Luigi Aloia[1], Bruno Di Stefano[1], Alessandro Sessa[2], Lluis Morey[1], Thomas Graf[1], Vania Broccoli[2], Luciano Di Croce[1]
[1]Gene Regulation, Stem Cells and Cancer, Center for Genomic Regulation (CRG), Barcelona, Spain, [2]Stem cells and Neurogenesis Unit, Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy

We have previously reported that the Zuotin-related factor, Zrf1, is able to de-repress developmental genes epigenetically silenced by the Polycomb group (PcG) of proteins. Epigenetic regulation mediated by PcG of proteins is crucial for embryonic development and stem cell features such as differentiation of pluripotent embryonic stem cells (ESC). To date molecular mechanisms regulating ESC differentiation are still largely unknown. Here we report that Zrf1 drives differentiation of mouse ESC toward multipotent neural progenitors (capable to give rise to neurons and glia). Furthermore, Zrf1 depletion in the ventricular zone of the embryonic cortex impairs the maintenance of neural progenitors in vivo. Molecular characterization indicates that Zrf1 is able to maintain self-renewal of neural progenitors by fine-tuning Wnt signalling. Indeed, Zrf1 is required to induce the expression of several Wnt ligand genes, through chromatin displacement of PcG thereby sustaining their proper expression in neural progenitors. Finally, we show that Zrf1 is essential for Sox2-driven reprogramming of mouse embryonic fibroblasts (MEFs) into induced neural stem cells (iNSCs) thus making it a potential target for regenerative medicine. Therefore, we have identified Zrf1 to be a crucial player involved in the specification of neural progenitors and in the maintenance of their features.

F-2381

HUMAN EMBRYONIC STEM CELL-DERIVED SEROTONERGIC NEURONS

Susanne M. Ström[1], Athina Samara[2], Orsolya Kreneisz[3], Joel C. Glover[1]
[1]Oslo University Hospital - Rikshospitalet, The Norwegian Center for Stem Cell Research, Oslo, Norway, [2]Department of Physiology, Institute of Basic Medical Sciences, University of Oslo, Norway, [3]Sars International Center for Marine Molecular Biology, Bergen, Norway

Serotonin is a major neuromodulatory neurotransmitter in nearly all animal species. In mammals, the physiological effects of serotonin range from regulation of subcortical sensory and motor systems, such as pain and the central pattern generator for locomotion, to regulation of higher cognitive functions, such as mood, awareness, consciousness and sleep. Deregulation of serotonin is implicated in a myriad of neuropathologies, including pain syndromes such as migraine, motor, sleep, eating and mood disorders.
Serotonergic neurons derived from human embryonic stem cells (hESCs) would provide a useful platform for standardized and quantitative studies of differentiation, synaptic function and pathogenic mechanisms.
OBJECTIVE
To modify existing protocols for serotonergic differentiation of hESCs to obtain physiologically differentiated serotonergic neurons.
METHODS
Cell culture and neuronal differentiation: In brief, H9 and HS360 hESC lines were cultured on human foreskin fibroblasts in 20% KO-SR in KO-DMEM supplemented with bFGF.
Neural differentiation was induced by forming embryoid bodies (EBs). EBs were plated on a mixture of matrix proteins and a 6-week protocol was followed wherein cultures were supplemented with combinations of cAMP, SHH, FGF4, FGF8, BDNF, AA, NT3 and NT4 to attain serotonergic differentiation.
Immunocytochemistry (ICC) was used to show the protein expression of early neural differentiation markers (Nestin and Tuj1) and mature neuronal markers (MAP2, NFH) as well as serotonergic phenotype-related markers (serotonin and Tph2).
Real time RT-PCR was performed to analyze multipotency and serotonergic phenotype-specific genes.
Western Blot (WB) was used to quantitate neuronal precursor and biogenic amine synthetic pathway proteins.
Patch clamp: Whole cell patch clamp recordings were performed on individual cells. Cells were filled with biocytin during recording to identify them in subsequent ICC.
RESULTS

The protocol promoted differentiation of human serotonin-containing neurons from hESCs. The cells expressed an increasing level of serotonergic phenotype-related genes along with markers of neuronal maturation, and voltage gated channels as seen by qRT-PCR. They also expressed the respective proteins, as demonstrated by ICC and WB. Voltage clamp recordings revealed both a voltage-gated transient inward current and a slow activating outward-rectifying current reminiscent of Na_V (principal current during action potential initiation) and the delayed rectifier K_V (principal current during repolarization), respectively. Current clamp recordings demonstrated overshooting action potentials.

DISCUSSION

The differentiation protocol used here promotes differentiation of hESCs to serotonin-containing neurons that express an appropriate set of serotonergic phenotype-related genes and proteins, as well as electrogenic properties conferring physiological function. These neurons can now be used to address many aspects of normal and pathological function in human serotonergic neurons.

F-2382

INSULIN CONCENTRATION-DEPENDENT CELL DEATH IN HUMAN NEURAL STEM CELL AND NEURON CULTURE

mi-yoon chang, Yong-Hee Rhee, Minyoung Choi, Hyun-Seob Lee, Sang-Hoon Yi, Sang-Mi Kim, Chang-Hwan Park, Sang-Hun Lee
hanyang university,Biomedical research institute, seoul, Korea, Republic of

Cultures of neural stem cells (NSCs) of human origins are useful in providing direct information to understanding human brain development and allows for the development of therapies for intractable human brain disorders. Human NSC (hNSC) cultures, however, are not commonly used, mainly due to the difficulty in consistently maintaining the cells in a healthy state. In this study, we show that hNSC cultures, unlike NSCs of rodent origins, are extremely sensitive to insulin, an indispensible culture supplement, and that a large portion of difficulties previously encountered in culturing hNSCs is likely due to being unaware of this insulin sensitivity. Like other neural cell cultures, insulin is absolutely required for hNSC cultures, as withdrawal of insulin supplementation resulted in massive cell death and retarded cell growth. On the other hand, severe apoptotic cell death was also detected in insulin concentrations optimized to rodent NSC cultures. Thus, healthy hNSC cultures were only attained in a narrow range of relatively low insulin concentrations. The mechanism underlying the cell death at high insulin concentrations was insulin resistance, in which cells became less responsive to insulin, resulting in a reduction in the activation of thePI3K-Akt pathway, responsible for a major intracellular cell survival signaling. The insulin-mediated cell death was manifested not only in all human NSCs tested, regardless of their origins, but also in differentiated human neurons. In addition to the practical applications in regards to human neural cell culturing, the findings of this study provide a meaningful explanation for the epidemiologic data demonstrating increased prevalence of neurodegenerative disorders in hyperinsulinemic type II diabetic patients.

This work was supported by a grant from the Bio and Medical Technology Development Program (2011-0019404), the Medical Research Center (2011-0028264) and (2012010261) funded by the National Research Foundation of Korea (NRF) of the Ministry of Education, Science and Technology (MEST), Republic of Korea

F-2383

DERIVATION AND FUNCTIONAL CHARACTERIZATION OF MULTIPOTENT RENAL PRECURSOR CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Min-Yong Kang
KAIST, Daejon, Korea, Republic of

We developed the novel protocol of the differentiation of human pluripotent stem cells (ESCs and iPSCs) toward functional renal precursor cells based on the serum and feeder free condition with controlling signaling pathway. We developed stage-specific method for the efficient differentiation by using chemically defined media with several exogenous proteins. hESCs and hiPSCs were consecutively differentiated through primitive streak, intermediate

mesoderm and metanephric mesenchyme, which stages was confirmed by q-PCR and immunostaining. At the end of the stage, we tested the multipotency of derived renal precursor cells and demonstrated that it could be differentiated into nephron consisting cells including renal tubular epithelial cells and podocytes in addition into other mesoderm lineage cells such as adipocytes and osteocytes with defined culture medium in vitro. In conclusion, we reported for the first time stage-specific protocol for the multipotent renal precursor cells derived from human ESCs and iPSCs with functional characterization in vitro and these results suggested its potential for the cell replacement therapy to support conventional medicine for the renal failure in the future.

F-2384

LARGE SCALE GENERATION OF MATURE MEGAKARYOCYTES FROM HUMAN PLURIPOTENT STEM CELLS BY A FORWARD PROGRAMMING APPROACH

Thomas Moreau[1], Amanda Evans[1], Matthew Trotter[2], Meera Arumugam[1], Maria Colzani[1], Willem Ouwehand[1], Roger Pedersen[2], Cedric Ghevaert[1]

[1]Haematology, University of Cambridge/NHSBT, Cambridge, United Kingdom, [2]Surgery, University of Cambridge, Cambridge, United Kingdom

Bone marrow Megakaryocytes (MKs) are the precursors of circulating blood platelets uniquely responsible for the tightly regulated process of blood clotting and maintaining homeostasis of the haematopoietic system. Due to the paucity of mature MKs in primary samples, the study of human MK biology has relied on in vitro differentiation systems from limited sources of haematopoietic progenitors or transformed cell lines. The ability to efficiently differentiate human pluripotent stem cells (hPSCs) towards MKs would be a great asset for basic research development as well as an exciting opportunity for in vitro production of genetically defined platelets which could ultimately revolutionise the field of transfusion medicine.
We have developed a novel approach we call forward-programming for production of MKs from hPSCs based on ectopic expression of transcription factors (TFs). Importantly, the protocol has been devised in chemically defined conditions and standardised using forced aggregation of single cells into embryoid bodies to aid transfer to fully controlled production systems (GMP). Through a transcriptome and protein interactome guided process, we first established a rank of TF candidates and from these identified a minimal combination of three factors - GATA1, FLI1 and TAL1 - as responsible for efficient production of MKs when expressed in hPSCs. The protocol leads to a robust production of pure population of MKs from a variety of hPSC lines with a mature MK yield in excess of fifty times the initial input in 20 days. Forward programmed MKs notably express key surface complexes associated with platelet function (GPIIb/IIIa, GPIb/V/IX, GPVI), show ultra-structures characteristic of the MK lineage and highly similar genome wide expression profiles compared to cord blood and peripheral blood derived MKs. Importantly, iPSC-derived MKs form proplatelets in vitro and in vivo experiments are in progress for further analysis of platelet release and function.
Reliable differentiation protocols are critical to achieve the potential of hPSCs in both basic research and clinical applications. The MK forward programming outcome surpasses existing protocols offering wider perspectives in using hPSCs for MK biological studies. We are currently establishing a collection of genetically engineered hPSC lines which should help getting a better understanding of novel gene function in MK and platelet deficiencies. In addition, concurrent ongoing work on development of physiologically relevant functionalised three dimensional scaffolds and bioreactors should bring us a step closer towards in vitro production of platelets for transfusion.

F-2385

STEMDIFF™ NEURAL INDUCTION SYSTEM FOR THE GENERATION AND MAINTENANCE OF NEURAL PROGENITOR CELLS FROM HUMAN PLURIPOTENT STEM CELLS USING AGGREGATE OR MONOLAYER CULTURE BASED METHODS

Vivian M. Lee, Alexandra Blak, Melanie A. Olson, Jennifer Antonchuk, Allen C. Eaves, Terry E. Thomas, Sharon A. Louis

STEMCELL Technologies, VANCOUVER, BC, Canada

Neural progenitor cells (NPCs) derived from human pluripotent stem cells (hPSCs) have become important tools to model human central nervous system (CNS) development and neurodegenerative disorders. We have previously demonstrated that the STEMdiff™ Neural Induction System can efficiently produce CNS-type NPCs from multiple human embryonic stem (hES) and induced-pluripotent stem (hiPS) cell lines. STEMdiff™ Neural Induction Medium (NIM) is a serum-free defined medium which was developed for use in conjunction with AggreWell™800 to form neural aggregates, which could later be propagated as neural rosettes. Recently, several studies have shown that neural induction from hPSCs can also be achieved in a monolayer-based culture system, wherein hPSCs are directly plated on a defined extracellular matrix and inductive factors are added to the media, without the use of feeder cells. The goals for the studies described here were: (1) To determine if STEMdiff™ NIM would also support efficient neural induction in a monolayer based culture system without aggregate formation, and (2) assess the expansion and properties of the NPCs obtained from either the AggreWell™800 aggregation or monolayer culture systems in our new STEMdiff™ Neural Progenitor (STEMdiff™ NP) Medium. For the first goal, hPSCs previously maintained in mTeSR1™ were seeded at 250,000 cells/cm^2 on polyornithine/laminin (PLO/L)-coated plates, and cultured in STEMdiff™ NIM for 9 days. Differentiation to NPCs was assessed at different time points by immunostaining cells in the monolayer cultures for the emergence of PAX6 expression with concomitant downregulation of OCT4. In the hES cell lines tested (H1, H9), approximately 25-30% of the cells were PAX6$^+$/OCT4$^-$ by day 3 and virtually all cells were PAX6$^+$/OCT4$^-$ by day 6 (n=5). Interestingly, in the hiPS cell lines tested, (WLS-4D1, A13700), neural induction was slightly delayed compared to the hES lines. Approximately 30-40% of hiPS cells were PAX6$^+$/OCT4$^-$ on day 5 and all cells were PAX6$^+$/OCT4$^-$ by day 8 or 9 (n=4). Overall, these data showed that STEMdiff™ NIM is highly effective at generating PAX6$^+$ NPCs in a monolayer culture protocol. To support further expansion of NPCs derived in STEMdiff™ NIM from aggregate- and monolayer-based cultures, we have developed STEMdiff™ NP Medium. Using the standard AggreWell™-based system, NPCs were generated, isolated with the STEMdiff™ Neural Rosette Selection Reagent, and plated in STEMdiff™ NIM as described previously. After 6-7 days, NPCs were harvested, dissociated into single cells then cultured at 100,000 cells/cm^2 on PLO/L coated plates in STEMdiff™ NP Medium. This procedure was repeated every 7 days for multiple passages. NPCs grown in STEMdiff™ NP medium exhibited 3-5 fold expansion over the first 3 passages (n=8), with minimal spontaneous neuronal differentiation (<10%) as determined by Tuj-1 expression. These NPCs could be propagated for more than 10 passages and expressed CNS-type NPC markers such as PAX6, SOX1, ZO-1, and NESTIN. Importantly, STEMdiff™ NP Medium also supported the growth of NPCs generated using the monolayer neural induction protocol described above, with comparable efficiencies. Overall, the STEMdiff™ Neural Induction System efficiently generates NPCs from hPSCs cells in either an aggregate or monolayer culture based system. Now, with the integration of new STEMdiff™ Neural Progenitor Medium into the workflow, further propagation of the hPSC-derived NPCs for many passages is possible.

F-2386

INSULIN GROWTH FACTOR 1 IS ABLE TO ORCHESTRATE FORMATION OF PRIMITIVE OCULAR STRUCTURES AND STRATIFIED NEURAL RETINA FROM HUMAN PLURIPOTENT STEM CELLS

Carla Mellough, Mahmoud Khazim, Joseph Collin, Evelyne Sernagor, David HW Steel, **Majlinda Lako**
Institute of Genetic Medicine, Newcastle University, Newcastle, United Kingdom

The recent acquisition of human induced pluripotent stem cell (hiPSC) technology has enabled new opportunities to create human models of development, function and disease. We and others have previously demonstrated that expandable populations of retinal progenitor, photoreceptor precursor and retinal pigmented epithelial (RPE) cells can be derived from human embryonic stem cells (hESC) and hiPSC. Recent major scientific advances in the field of stem cell differentiation have demonstrated the ability to generate stratified

neural retina containing multiple retinal phenotypes which present valuable models of development and disease and an excellent resource of transplantable cells. This phenomenon can occur spontaneously in low frequency, or in greater frequency under directed stage-specific differentiation conditions, however generation of the photoreceptor outer segment still remains elusive. We now report that the addition of a single factor, insulin-like growth factor 1 (IGF-1), to hESC/hiPSC cultures for the duration of differentiation can orchestrate the formation of ocular-like structures containing not only RPE and neural retina, but also various other elements of the developing eye including primitive lens and cornea. Laminar organisation of IGF-1-derived retina is reminiscent of developing human retina and comprises multiple phenotypes including photoreceptors, bipolar, amacrine and ganglion cells, which established synaptic connections and led to the formation of a visible plexiform layer. Primitive rod- and cone-like photoreceptor inner and outer segments were observed and hESC/hiPSC-derived photoreceptor cells show functional properties similar to native photoreceptor cells, making these derivatives excellent candidates for cell replacement studies in addition to *in vitro* retinal disease modelling.

F-2387

PRODUCTION OF FUNCTIONAL BETA CELLS FROM HUMAN PLURIPOTENT STEM CELL-DERIVED PANCREATIC PROGENITORS

Yuki Ohi, Alan Agulnick, Anindita Bhoumik, Kuniko Kadoya, Mark Moorman, Evert Kroon, Kevin D'Amour, Olivia G. Kelly
ViaCyte, Inc., San Diego, CA, United States

The mission of ViaCyte, Inc. is to create a therapy for diabetes that employs both stem cell technology and an encapsulation medical device (Encaptra® drug delivery system). We have previously shown that pancreatic progenitors derived from human embryonic stem cells (hESC) efficiently generate glucose-responsive, insulin-secreting cells after engraftment into mice using several formats (Kroon et al. 2008, Nature Biotech; Kelly et al. 2011, Nature Biotech.), including a scalable suspension-based system that was recently published for our clinical CyT49 hESC line (Schulz et al. 2012, PloS One). We explored using this new system with other human pluripotent stem cells, including induced pluripotent stem cells (iPSC; lines provided by Cellular Dynamics). In brief, using an optimized version of our four-stage suspension-based protocol, we reproducibly differentiated hESC and hiPSC to cell populations highly enriched for pancreatic cell lineages. Evidence of directed differentiation is shown at both the mRNA and protein level at each stage of endodermal and pancreatic intermediate, and by flow cytometry of the final population. After implantation into immune-compromised mice, encapsulated hESC- and hiPSC-derived pancreatic progenitors matured into glucose-responsive, insulin-secreting cells that were able to regulate blood glucose levels upon streptozotocin (STZ) ablation of mouse beta cells. In summary, our technology for producing functional beta cells is adaptable for a variety of pluripotent stem cell lines, and is the first demonstration of iPSC-derived beta cells correcting glycemia in a diabetes animal model.

F-2388

HTT AFFECTS DIFFERENTIATION OF HES CELLS BY MODULATING TGFβ SIGNALING

Ismail Ismailoglu, Aryeh Warmflash, Joanna Krzyspiak, Ali Brivanlou
The Rockefeller University, New York, NY, United States

Neurodegenerative Huntington's Disease (HD) is caused by an extension of the poly-glutamine tract at the N-terminal of Huntingtin (Htt) protein. The most devastating effects of HD appear in the brain and later in life, but Htt is expressed ubiquitously during development. Signifying the importance of Htt for early development, Htt-/- mouse

embryos die by E8.5 with defects in gastrulation. In this study, we aimed to discover the role Htt plays during development using human embryonic stem cells as a model. By employing knockdown and overexpression strategies, we have discovered a novel connection between the Htt protein and TGFβ signaling. We showed that knockdown of Htt protein in hES cells causes an increase in response to signaling that affects both Smad1 and Smad2 dependent arms of the pathway. This increase was reversed by ectopic expression of wild-type or long-polyQ Htt. Knockdown of Htt also increased the differentiation of hES cells to Bra+ mesendoderm cells after BMP4 treatment. Based on these observations we propose that primitive streak development is defective in Htt knockout embryos due to increased response from primitive streak cells to BMP4 signal.

F-2391

CONTROLLING ESC FATE AND MORPHOGENESIS IN VITRO VIA HYDROGEL MICROFLUIDICS

Steffen Cosson, Matthias P. Lutolf
Institute of Bioengineering, EPFL, Lausanne, Switzerland

Biomolecular signaling is of upmost importance in governing the patterning of the developing embryo where morphogens regulate key cell fate decisions such as lineage specification. In vivo, these factors are presented in a spatiotemporally tightly controlled fashion and in the context of a soft and hydrated microenvironment. Traditional in vitro methods fall short of recapitulating this complex physiological morphogen display. Emerging microfluidic technologies based on poly(dimethylsiloxane) (PDMS) now allow the delivery of biomolecules within microchannels with very high spatiotemporal precision. However, state-of-the-art PDMS microchips are far from ideal for stem cell culture due to issues such as medium evaporation, limited space for cell growth, shear stress and a non-physiological microenvironment that might adversely impact stem cell fate. As a result, microfluidic cell culture systems are not yet suitable to unravel complex multicellular phenomena.
To address these limitations, we have been developing hydrogel-based microfluidic systems that can decouple macro-scale cell culture on the gel surface from the precise spatiotemporal biomolecule delivery at the micro-scale, i.e. through the gel layer. The gel-embedded microfluidic networks can be perfused by hydrostatic pressure or syringe pumps, generating transient and stable gradient with high precision. The hydrogel chips, used here as simple inserts that are compatible with conventional multiwell plate formats, were bioengineered to afford mouse embryonic stem cell (ESC) culture in both adherent format and as uniformly sized embryoid bodies (EBs). We validated this system by spatially controlling neural fate commitment of Sox1-GFP ESCs upon retinoic acid delivery. This technique should be broadly applicable for testing the effect of the dose and timing of morphogens, singly or in combination, on stem cell fate. Perhaps even more interestingly, our platform allows the generation of morphogen gradients across individual EBs to study more complex morphogenetic processes.

F-2392

A NOVEL VIDEO AND ESC-BASED ANALYSIS TO DETERMINE TERATOGENIC EFFECTS OF CONVENTIONAL AND HARM-REDUCTION TOBACCO PRODUCTS ON HUMAN BONE DEVOLPMENT

Ivann K.C. Martinez, Henry Affeldt III, Nicole R.L. Sparks, Prue Talbot, Bir Bhanu, Nicole I. zur Nieden
University of California, Riverside, Riverside, CA, United States

In the United States, it has been estimated that 1 out of 5 people smoke. Numerous studies have demonstrated that the use of tobacco products can lead to cancer, respiratory complications, as well as cardiovascular diseases. Additionally, the use of tobacco products can increase the risk for osteoporosis. Moreover, several studies have identified that chemicals found in cigarettes and their smoke can decrease bone healing and hinder the formation of new osteoblasts. While these smoke-induced effects on the adult skeletal system are widely accepted, its effect on the formation of the embryonic skeleton is largely unknown.
Here, we aimed to compare commercially available conventional and harm reduction tobacco products to determine their effects on bone development in vitro using human embryonic stem cells undergoing osteogenesis. Specifically, the efficacy of mainstream smoke obtained from the filter end of the cigarette was compared to that of

sidestream smoke from the burning end of the cigarette. A cytotoxic dose response curve was generated by an MTT assay. Furthermore, a novel video-based analysis of osteogenesis in real-time was employed to compare the level of calcification in response to the two types of smoke generated by the conventional and harm-reduction tobacco products.
Our results suggest that teratogenic effects on osteogenic differentiation were attributable to addition of whole smoke from tobacco products. In addition, sidestream smoke was found to be more cytotoxic than mainstream smoke. Moreover, this study suggests that some harm-reduction products may have less of a detrimental impact on osteogenesis than their conventional counterparts, while others significantly reduce calcification in doses that are not cytotoxic. Furthermore, video-based analysis can be used to determine calcification in culture during development. In its totality, this study demonstrates that video-based analysis is a viable alternative to chemical based assays of differentiation to determine the teratogenic effects of environmental toxicants, among them cigarette smoke, on human bone development.

F-2393

A CELLULAR MODEL FOR MUSCULAR DYSTROPHY USING AFFECTED HUMAN EMBRYONIC STEM CELLS

Leslie Caron, Miriam Gubernator, Simer Khaira, **Uli Schmidt**
Genea Stem Cells, Sydney, Australia

Muscular dystrophies represent a major unmet clinical need, arising from the loss of functional muscle cells. At present, little is known about muscle diseases and no treatment is available specifically for muscular dystrophy. Developing human cellular models is critical not only for understanding the molecular disease mechanism, but can also have a major impact on the development of new therapies. A major challenge in drug development for muscular dystrophy is the identification of model systems that accurately recapitulate normal and diseased human muscle physiology in vitro. Because of their plasticity and unlimited capacity to proliferate, human embryonic stem cells (hESCs) potentially represent a renewable source of skeletal muscle cells (SkMCs). The availability of disease-affected stem cells is the first step towards a muscular disease model. At Genea Stem Cells, we have derived several hESC lines carrying the chromosomal mutations responsible for different muscular dystrophies including Facio-Scapulo-Humeral Muscular Dystrophy (FSHD), Becker Muscular Dystrophy, Merosin Deficiency 1A, Nemaline Myopathy 2 and Myotonic Dystrophy I and II. The next critical step is to differentiate hESC into SkMCs, the affected cell type. Currently, SkMC derivation from hESCs only achieves low yields and requires serum, genetic manipulations and the generation of 3D embryoid bodies. To produce SkMCs in a defined monolayer system, we performed a series of high-throughput screens to determine optimal conditions for the target pathway and establish a simple and rapid protocol to efficiently generate SkMCs in vitro. Using our well-established stem cell differentiation discovery platform utilizing robotics and high-content analysis, we screened an extensive compound library of regulators of cell signalling pathways for modulators of the myogenic differentiation process. In our novel protocol for SkMC differentiation, hESCs are induced to form MyoD+ myoblasts using a combinatorial application of growth factors and small molecules. This intermediate population is further differentiated into a population of mature and multi-nucleated myotubes, which display contractile ability and express the muscle specific markers Myogenin, Desmin and sarcomeric MHC. In conclusion, we show that within 28 days fully functional SkMCs can be derived from hESC in an efficient, reproducible and defined manner. This discovery combined with our bank of disease-affected stem cell lines enables us to produce disease-specific human SkMCs with broad applications in modelling muscular diseases, contributing to a better understanding of muscle disease mechanisms that will ultimately assist in the discovery and development of effective treatments for muscular dystrophies.

F-2394

NANOPARTICLES MODULATE HUMAN EMBRYONIC STEM CELL DIFFERENTIATION

Shyr-Yeu Lin[1], Cheng-Hung Wei[2], Yu-Tien Chiu[2], Shih-Chung Wang[2], Kuo-Chu Hwang[3]

[A]Department of Obstetrics and Gynecology, [B]Department of Medical Research, [1]Mackay Memorial Hospital, Taipei, Taiwan, [2]Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan

Introduction: The field of studying nanoparticles is fast developing. The major focus of it is on the medical application. However, the effects of nanoparticles on the various types of cells in the body should be carefully defined before clinical uses. Since human embryonic stem cells (hESCs) can differentiate into all kinds of the cells in the body, it may be used as an in vitro platform to understand the effects of nanoparticles on human cells. Also, the appropriate nanoparticles may be a good tool to use in directed differentiation of hESCs for cell therapy in the future. Thus, as a first step, we aim to study on the effects of 8 types of nanoparticles on hESC differentiation. **Methods:** The nanoparticles we targeted are TiO_2, TiO_2 (P25), TiO_2 fiber, Au, Ag, Fe, ND acid wash and ND graphtized. The hESCs were maintained on the MEF (mouse embryonic fibroblasts) feeder layer. The hESC colonies were cut into 8 ~ 10 pieces per colony, then put into suspension culture to induce EB (embryoid body) formation. Various nanoparticles were added into differentiation culture system. Shaking the cultures was performed to allow even distribution of particles and contacts between EBs and nanoparticles. The time points of analyses were set on day 7, day 18 and day 27. Germ cell, endodermal, mesodermal and ectodermal lineages were determined by immunohistochemistry targeting DDX4, Sox17, Foxa2, Brachyury, Desmin, Notch1 and Pax6. The quantification of gene expression and positive events were performed by FACS-like tissue cytometry. **Results:** In general, nanoparticles facilitate and promote hESC differentiation than the controls. Based on the positive event percentages and protein expressions in the whole culture cells, TiO_2 (P25) seems to facilitate germ cell lineage; Ag, Au, TiO_2 (P25) and ND, endodermal lineages; TiO_2 and TiO_2 (P25), mesodermal lineages; TiO_2 (P25) and ND, ectodermal lineages. **Conclusions:** The study provides a data base for the effects of 8 nanoparticles on hESC differentiation.

F-2395

APPLICATION OF NANOTECHNOLOGY TOOLS TO GENERATE LTA-PDMS TRANSPARENT GRIDS FOR TIMELAPSE MONITORING OF EMBRYOID BODY FORMATION THAT IMPROVES EVALUATION OF HESCS AND ENABLES DIRECTED TISSUE ENGINEERING

Martin L. Tomov, Zachary T. Olmsted, Janet L. Paluh
Nanobioscience, College of Nanoscale Science & Engineering, UAlbany SUNY, Albany, NY, United States

Embryoid bodies (EBs) provide a three dimensional (3D) precursor for lineage differentiation used frequently in evaluating pluripotency of stem cell lines. They are also being applied more often to the generation of organoids and tissue engineering. Spontaneously formed EB populations in free suspension are not ideal for evaluation of stemness nor for tissue engineering strategies since they are generally of mixed sizes, shapes and may contain fused aggregates, all of which complicate interpretations and use. Here, we describe a method for generation of uniform EBs from human embryonic stem cells (hESCs) that are of tunable size and shape in a transparent template that also permits time-lapse tracking of the rate of EB formation. This method includes production of lithography-templated arrays (LTA) and PDMS spin-coating of these templates (LTA-PDMS) to produce a transparent user defined grid that can be seeded with 2D cell clusters or 3D early forming aggregate hESC cells. This method generates uniform EBs following live tracking for EB retrieval and differentiation for statistical evaluation. This lithography-based method offers the opportunity for massively parallel throughput of EBs of controllable parameters (shape, depth and diameter). The stem cell pluripotency of LTA-PDMS cultivated EBs was evaluated for differentiation along ectoderm, endoderm, and mesoderm lineages. Our method successfully cultivates uniform EBs capable of differentiating along the three germ layers. Further, in this study we show that human EBs formed in 200 μm wells initiate differentiation more rapidly and uniformly than EBs from 500 μm wells or those formed freely in suspension as larger fused EBs. Our approach offers an informative new method for analysis of hESCs through the study of their 3D cell aggregate EBs and enables statistical monitoring, and dimensional control of cell aggregate formation with implications for tissue engineering, tumor cell analysis, and regenerative medicine applications.

F-2396

NEURAL DIFFERENTIATION OF IPS CELLS FROM THE COMMON MARMOSET (CALLITHRIX JACCHUS).

Steven L. Farnsworth[1], Anuja Mishra[2], Peter J. Hornsby[1]
[A]Physiology, [B]The Barshop Institute for Longevity and Aging Studies, [1]University of Texas Health Science Center at San Antonio, San Antonio, TX, United States

Induced pluripotent stem (iPS) cells are an important stem cell population to potential regenerative medicine applications. Here we utilize iPS cells derived from the common marmoset (Callithrix jacchus) monkey to assess neuralization potential by pharmacological inducers of neural differentiation. iPS cell pluripotency depends on active FGF and activin/nodal/TGF-β signaling, among other factors. Upon removal of iPS cells from pluripotency-supportive conditions containing bFGF and activin, differentiation can be initiated. Approach: To direct differentiation in the neural lineage, we utilized an embryoid body generation assay in combination with a cocktail treatment of active small molecules: DMH1 (BMP inhibitor), SB431542 (activin/nodal/TGF-β inhibitor), BIO (GSK-3β inhibitor), Y-27632 (ROCK/Rho inhibitor) and all-trans retinoic acid. Results: After 6 days of treatment in the absence of bFGF and GSK-3β inhibitor, we found significant increases in the mRNA levels of neural markers NCAD (30-fold increase, P = 0.0001) and ERBB3 (12-fold increase, P = 0.0129). We found that bFGF addition (at 2 ng/mL) had an inhibitory effect on NCAD/ERBB3 induction. When GSK-3β inhibitor (BIO) was included, NCAD mRNA levels further increased (from a 34- to 102-fold increase over baseline, P = 0.0001), as was ERBB3 (34-fold, P = 0.0035). bFGF withdrawal also increased FOXA2 levels (endoderm marker), but we report that inclusion of the GSK-3β inhibitor (BIO) ablated induction from a 15-fold increase to 3-fold increase, P = 0.0318). Conclusions: These data indicate that FGF signaling is inhibitory to neuroectoderm differentiation, while GSK-3β inhibition promotes neuralization of marmoset iPS cells as indicated by NCAD and ERBB3 levels. Neuralization is an important initial step towards in vitro generation of neuroectoderm epithelial cells, allowing for future derivation of neural progenitor or neural crest cells. Here we report that iPS cells derived from the common marmoset respond to pharmacological inducers of neural differentiation. These studies are important for the further development of the marmoset as a preclinical model for future testing of iPS-mediated autologous cell therapies.

F-2397

PROTEIN KINASE C REGULATES HUMAN PLURIPOTENT STEM CELL SELF-RENEWAL

Masaki Kinehara, Suguru Kawamura, Daiki Tateyama, Mika Suga, Hiroko Matsumura, Sumiyo Mimura, Mitsuhi Hirata, Kana Yanagihara, Furue Miho K.
National Institute of Biomedical Innovation, Osaka, Japan

The self-renewal of human pluripotent stem (hPS) cells including embryonic stem and induced pluripotent stem cells have been reported to be supported by various signal pathways. Among them, fibroblast growth factor-2 (FGF-2) appears indispensable to maintain self-renewal of hPS cells. We previously developed a growth factor defined serum-free medium hESF9 for human ES cell culturing without feeder cells. In this culture conditions, we found that heparin enhances endogenous FGF-2 activity. FGF-2 induces the phosphorylation of mitogen-activated protein kinase/extracellular signal-regulated kinase-1/2 (ERK-1/2) kinase (MEK), phosphatidylinositol-3 kinase (PI3K)/AKT, and glycogen synthase kinase-3 (GSK-3) pathway in hPS cells. However, the contributions of FGF-2 downstream pathways in the self-renewal of hPS cells have been controversial
Then, we screened a kinase inhibitor library using a high-throughput alkaline phosphatase (ALP) activity-based assay to understand FGF-2-related molecular mechanisms regulating self-renewal of hPS cells. We found that an inhibitor of protein kinase C (PKC), GF109203X (GFX) increased ALP activity in hPS cells. GFX inhibited FGF-2-induced phosphorylation of glycogen synthase kinase-3β (GSK-3β), suggesting that FGF-2-induced PKC inhibited the activity of GSK-3β. GFX negated differentiation of hPS cells induced by the PKC activator, phorbol 12-myristate 13-acetate whereas Gö6976, a selective inhibitor of PKCα, β, and λ isoforms could not counteract the effect of PMA. Intriguingly, functional gene analysis by RNA interference revealed that the phosphorylation of GSK-3β was reduced by siRNA of PKCδ, PKCε, or ζ. The phosphorylation of ERK-1/2 was reduced by siRNA of PKCε or ζ whereas the phosphorylation of AKT was reduced by PKCε in hPS cells.

In conclusion, our study suggested that FGF-2 induces PI3K/AKT and MEK/ERK-1/2, but also PKCs in hPS cells. PI3K/AKT promotes cell self-renewal whereas the MEK/ERK-1/2, PKC/ERK-1/2 and PKC/GSK-3β pathways down-regulate hPS cell self-renewal. Our study may uncover a novel key signaling pathway in regulating the hPS cell self-renewal and differentiation.

F-2398

HESC DERIVED DOPAMINERGIC NEURONS AS A PLATFORM FOR HIGH-THROUGHPUT SCREENING ASSAY

Talya Mordecai-Daniel[1], Ofer Wiser[2], Benjamin Reubinoff[3]
[1]The Sidney and Judy Swartz Human Embryonic Stem Cell Research Center, The Goldyne Savad Institute of Gene Therapy, Hadassah-Hebrew University Hospital, Jerusalem, Israel, [2]BioPark Building Suite 500, CellCure Neurosciences Ltd, Jerusalem, Israel, [3]The Sidney and Judy Swartz Human Embryonic Stem Cell Research Center, The Goldyne Savad Institute of Gene Therapy, and The Department of Obstetrics and Gynecology, Hadassah-Hebrew University Hospital, Jerusalem, Israel

Parkinson's disease is characterized by degeneration of dopaminergic (DA) neurons in the substantia nigra, resulting in movement abnormalities, rigidity and tremor. The symptoms can be transiently alleviated by *L*-DOPA and other drugs (i.e., dopamine receptor agonists); nevertheless, it has so far not been possible to stop the progression of the disease. Therefore, it is of great interest to identify novel compounds with neuroprotective and regenerative properties for the treatment of Parkinson's disease.
Screening of large libraries for compounds with potential therapeutic effects has not been done in mammalian animal models of Parkinson's disease due to high costs and time limitations. Hence, the development of a cell-based high-throughput screening (HTS) system to identify novel therapeutic compounds would be highly valuable.
Here, we exploited the potential of human embryonic stem cells (hESCs) as a renewable source of DA neurons, to serve for the development of HTS assay for molecules which can affect the survival and function of DA neurons.
In order to direct the differentiation of hESCs into DA neurons, the cells were first directed to become neural progenitors within free-floating spheres in the presence of the dual SMAD inhibitors, FGF8, Purmorphamine and CHIR. They were further directed to differentiate into DA progenitors as adherent cultures in the presence of FGF8, Purmorphamine and CHIR. Finally, they were differentiated into mature DA neurons in the presence of survival factors.
We then developed a novel system for the detection of functional live DA neurons *in vitro*. This system is based on the specific binding and uptake of the fluorescent ligand DansylD1 (dopamine labeled with dansyl molecule) by dopamine transporter (DAT), expressed on the surface of DA neurons.
We used a fluorescence microscope and a fluorescence micro-plate reader to detect and quantify the uptake levels of the DansylD1 fluorescent ligand molecule by mature DA neurons.
For calibration and qualification of the DansylD1 detection system we first used PC-12 cells as positive control of dopaminergic cells expressing DAT. We used specific blockers to reduce nonspecific background fluorescence related to binding of DansylD1 to dopamine receptor D2, which is not exclusively expressed on DA neurons.
We have demonstrated that DansylD1 specifically labeled hESC-derived differentiated cells, which were immunoreactive with anti-Tyrosin Hydroxylase (TH), a DA neuron marker. In addition, after incubation with the DA neuron-specific toxin, 6-OH-dopamine, micro-plate reader showed reduction in the fluorescent signal of DansylD1-labeled cells.
Our results pave the way for HTS of agents on human pluripotent-derived DA neurons in order to find compounds with neuroprotective or regenerative properties that could potentially be further used for the treatment of Parkinson's disease.

F-2401

THE ROLE OF THE ABCG2 ATP-BINDING CASETTE TRANSPORTER IN HUMAN EMBRYONIC STEM CELLS

Raji Padmanabhan[1], Kevin G. Chen[2], Jean-Pierre Gillet[1], Misty Handley[1], Barbara S. Mallon[2], KyeYoon Park[2], Rebecca S. Hamilton[2], Pamela G. Robey[3], Ronald G. McKay[4], Michael M. Gottesman[1]

[A]CCR, NCI, [B]Stem Cell Unit, NINDS, [C]NIDCR, [1]NIH, Bethesda, MD, United States, [2]Lieber Institute, JHU, Baltimore, MD, United States

The expression and function of several multidrug transporters (including ABCB1 and ABCG2) have been studied in human cancer cells and in mouse and human adult stem cells. However, the expression of ABCG2 in human embryonic stem cells (hESCs) remains unclear. Limited and contradictory results in the literature from two research groups have raised questions regarding its expression and function. In this study, we used quantitative real-time PCR, Northern blots, whole genome RNA sequencing, Western blots, and immunofluorescence microscopy to study ABCG2 expression in hESCs. We found that full-length ABCG2 mRNA transcripts are expressed in undifferentiated hESC lines. However, ABCG2 protein was undetectable even under embryoid body differentiation or cytotoxic drug induction. Moreover, surface ABCG2 protein was coexpressed with the differentiation marker stage-specific embryonic antigen-1 of hESCs, following constant BMP-4 signaling at days 4 and 6. This expression was tightly correlated with the downregulation of two microRNAs (miRNAs) (i.e., hsa-miR-519c and hsa-miR-520h). Transfection of miRNA mimics and inhibitors of these two miRNAs confirmed their direct involvement in the regulation ABCG2 translation. Our findings clarify the controversy regarding the expression of the ABCG2 gene and also provide new insights into translational control of the expression of membrane transporter mRNAs by miRNAs in hESCs.

F-2402

CHARACTERISATION OF HUMAN EMBRYONIC STEM CELL DERIVED PANCREATIC BETA CELLS

Jacqueline V. Schiesser[1], Suzanne J. Micallef[2], Emma M. Allister[3], Alexandre B. Hardy[3], Michael B. Wheeler[3], Andrew G. Elefanty[1], Edouard G. Stanley[1]

[1]Murdoch Childrens Research Institute, Parkville, Australia, [2]Monash University, Clayton, Australia, [3]Department of Physiology, University of Toronto, Toronto, ON, Canada

Human Embryonic Stem Cells (hESCs) are immortal, pluripotent cells derived from the inner cell mass of the preimplantation embryo. These cells have the potential to differentiate into all of the cell types found in the human body, including insulin-producing beta cells. As such, hESCs have been advanced as a potential source of beta cells that could provide an alternative to cadaver-derived islets for the treatment of type I diabetes.
We explored factors affecting the in vitro generation and function of insulin producing beta cells using a hESC reporter line that expressed GFP under the regulatory control of the INSULIN locus. We found that in vitro derived INSULIN-GFP+ cells were a heterogeneous population that contained a substantial fraction of INSULIN+ cells that did not express either glucagon or somatostatin.
INSULIN-GFP+ cells isolated by flow cytometry were reaggregated to form clusters that were then cultured in vitro for up to 3 months. Examination of these clusters using immunofluorescence analysis indicated that INSULIN-GFP+ cells had the ability to form monohormonal cells representing the three most prominent endocrine lineages found in pancreatic islets: insulin, somatostatin and glucagon expressing cells. INSULIN-GFP+ cells retained following extended in vitro culture expressed transcription factors associated with beta-cell maturity, including NKX6.1.
Transmission electron microscopy of monohormonal INSULIN+ cells revealed the presence of hormone granules, some of which displayed a halo typical of mature insulin granules associated with islet-derived beta cells. Electrophysiology studies indicated that monohormonal INSULIN-GFP+ cells displayed a Kv current that resembled that of normal human beta cells and possessed an active Nav current.
INSULIN-GFP+ cells were analysed for their ability to respond to a variety of secretogogues known to induce C-peptide secretion in human beta cells, in addition to analysing for the ability to secrete C-peptide in response to glucose load. It was found that INSULIN-GFP+ cells were able to reliably secrete C-peptide in response to both KCl and IBMX, and variable C-peptide secretion was seen in response to glucose load.
In conclusion, we have established a protocol that results in the generation of a heterogeneous INSULIN+ cell population, that can be further cultured in vitro to form monohormonal endocrine cells of three different lineages found in the adult pancreas. Monohormonal INSULIN cells generated resemble human beta cells in regards to the pres-

ence of a Nav and Kv current, contain hormone granules, and are able to secrete C-peptide in response to secretogogues such as KCl and IBMX, and show varying ability to secrete C-peptide in response to glucose load.

Totipotent/Early Embryo Cells

F-2411

BIFURCATION ANALYSIS OF SINGLE-CELL GENE EXPRESSION DATA REVEALS EPIGENETIC LANDSCAPE

Eugenio Marco[1], Robert L. Karp[2], Guoji Guo[3], Lorenzo Trippa[1], Stuart H. Orkin[1], **Guo-Cheng Yuan**[1]
[1]Dana-Farber Cancer Institute, Boston, MA, United States, [2]Harvard Medical School, Boston, MA, United States, [3]Children's Hospital Boston, Boston, MA, United States

Waddington's epigenetic landscape has been widely accepted as a fundamental concept to describe the development process in complex systems. However, the molecular underpinnings of the epigenetic landscape remain poorly characterized. Here we present a novel mathematical approach to reconstruct the epigenetic landscape from single-cell gene expression data, combining dynamic clustering and bifurcation analysis. By analyzing two different datasets, obtained from mouse early embryos and bone marrow, respectively, we provide strong evidence that dynamic changes in gene expression during cell differentiation are highly organized and can be well described by the bifurcation theory. Characterization of the epigenetic landscape identified candidate regulators driving the initial events during cell differentiation, without relying on prior knowledge about the underlying gene regulatory network. Furthermore, we predicted the effect of perturbations of transcription factor expression levels on cell differentiation. Our molecular characterization of the epigenetic landscape may serve as a useful guide for future mechanistic studies of developmental regulation.

F-2412

RNF12 IS ESSENTIAL FOR X INACTIVATION IN FEMALE MOUSE EMBRYONIC STEM CELLS AND IS REQUIRED FOR FEMALE MOUSE DEVELOPMENT

Tahsin Stefan Barakat, J.Anton Grootegoed, Joost Gribnau
Reproduction and Development, Erasmus MC, Univ Medical Center Rotterdam, Rotterdam, Netherlands

X chromosome inactivation (XCI) in placental mammals is a mechanism which equalizes dosage of X-linked genes between females and males. This crucial process for female development is first initiated around the two-to-four cell stage in the pre-implantation embryo in an imprinted way, in which the paternal X chromosome is inactivated. In the inner cell mass (ICM) of the developing blastocyst, the inactivated X chromosome is reactivated, allowing a short window in time in which a double dosage of X-linked gene products is tolerated. Upon further development, random XCI is initiated in the post-implantation embryo, thereby coupling initiation of XCI to differentiation. Female mouse embryonic stem (ES) cells, which are derived from the ICM, are therefore characterized by two active X chromosomes, and undergo XCI upon differentiation, making them a unique *in vitro* model. Initiation of chromosome wide silencing of the X chromosome is regulated by the up-regulation of the non-coding RNA *Xist*. In undifferentiated cells, the core pluripotency factor network consisting of Nanog, Oct4 and Sox2 indirectly suppress Xist up-regulation. We have previously shown that Xist up-regulation is triggered by a stochastic mechanism, in which the X-linked gene *Rnf12* acts as an X-linked activator of XCI, allowing XCI initiation only in females. The encoded RNF12 is an E3 ubiquitin protein ligase, which activates *Xist* by targeting Rex1 for proteasomal degradation. Over expression of RNF12 results in ectopic XCI, and homozygous *Rnf12*-/- ES cells fail to undergo XCI upon differentiation, showing that RNF12 is a key factor in the XCI initiation process. Here we further decipher the XCI initiation mechanism, by showing that RNF12 is continuously required for maintaining XCI in female cells. To address the role of X chromosome pairing in the initiation of XCI, we have generated ES cell lines containing deletions of all known X-pairing elements. Surprisingly, these cell lines show normal XCI initiation. In addition, in experimental XX-XY heterokaryons, XCI initiation is also found in the male nucleus upon differentiation with normal kinetics, confirming that X-pairing is indeed not functional required for XCI. Instead, XCI initiation is regulated by *cis*-acting elements

located in the vicinity of the *Xist* locus, and *trans*-acting factors, of which we found RNF12 to be the most important. The generation of an *Rnf12* knockout mouse model confirms that RNF12 is also crucial for XCI initiation *in vivo*. Whereas male *Rnf12*-/Y are viable, female *Rnf12*-/- mice fail to undergo XCI leading to lethality in the post-implantation embryo. *Rnf12*-/+ animals inheriting the maternal knockout allele are lethal due to silencing of the paternal *Rnf12* allele upon imprinted XCI. *Rnf12*+/- females inheriting the paternal knockout allele are viable but show an XCI defect, with cells which have failed to undergo XCI properly. These cells with absence of normal dosage compensation are found in many tissues, without an obvious physiological phenotype. Therefore, these animals for the first time show that it is possible to live with cells with failure of dosage compensation, challenging the more than 50 year old dogma that XCI is essential for females. This will have important implications for understanding and treating X-linked diseases in the future, as it might become possible to treat X-linked diseases in females by reactivating an previously inactivated X chromosome.

Germline Cells

F-2421

FIBROBLAST GROWTH FACTOR RECEPTOR SIGNALING IN ADULT SPERMATOGONIAL STEM CELLS

Laura Andres-Martin, Nicholas Assif, Sam Sultan, Courtney Sachs, Marco Seandel
Surgery, Weill Cornell Medical College, New York, NY, United States

Spermatogonial Stem Cells (SSCs) in the adult testis support spermatogenesis and transmit genetic information to the offspring. Many studies suggest that changes in the niche affect stem cell maintenance and can dictate cell fate. Therefore, identification of signals emanating from the niche is critical to understanding stem cell function and to enable faithful, long-term propagation of adult SSCs in vitro. In vitro expansion of SSCs requires not only glial derived neurotrophic factor (GDNF) but also the addition of other factors including fibroblast growth factor (FGF2). FGF2 itself is insufficient to maintain SSCs in culture but together with GDNF, increases SSC proliferation. Sertoli cells, Leydig cells and differentiating germ cells produce FGF2 in the testis but very little is known about the specific roles of FGF signaling in SSC survival. Recent studies show that both exogenous FGF2 and endogenous FGF2 produced by germ cells promote self-renewal of SSCs in vitro. FGF2 can potentially bind to all 4 FGFRs. Moreover, different receptors isoforms regulated by alternative splicing exhibit opposite affinities for different FGF ligands. Strikingly, constitutional mutations in FGFRs that confer gain-of-function give rise to a large variety of human disease phenotypes. We are interested in the contribution of different FGFRs isoforms to SSC stemness and differentiation. Also, if FGFR signaling is critical for SSCs, it is possible that multiple FGF ligands play important roles in maintenance of SSCs in vivo and in vitro. Specifically, we hypothesize that shifts in growth factor receptor isoform expression correlate with changes in self-renewal capacity of SSCs. Using specific primers we have detected the expression of different FGFRs isoforms in multiple independent SSCs cell lines derived from adult mice. We have performed absolute quantification using real-time PCR to compare the levels of expression between the different isoforms. Among FGFRs, FGFR1 and FGFR3 are the most abundantly expressed receptors. Moreover, there is mutually exclusive expression of the two main isoforms for each receptor. Interestingly, we have detected expression at high levels of unusual transcripts of FGFRs that seem to be exclusively expressed in SSCs. These variants have been detected in human tissue previously but are poorly characterized. Microarray data and real time PCR experiments in murine SSCs vs. feeders reveal differential expression for some FGFs. For example, FGF9 is highly expressed in SSCs comparing to JK1 feeder cells while FGF2 is predominantly expressed by JK1 feeder cells compared to SSCs. We have performed in vitro differentiation assays using retinoic acid to study changes in FGFR signaling. Preliminary mRNA expression data indicate that FGFR1 and FGFR2 isoforms are more abundant in SSCs, while FGFR3 isoforms are more expressed following exposure to retinoic acid. Based on our current model, different FGF ligands binding to specific FGFRs isoforms could influence SSC fate in the adult testis.

F-2422

CRYOPRESERVATION OF PORCINE SPERMATOGONIAL STEM CELLS BY SLOW-FREEZING IN TREHALOSE

Yong-An Lee[1], **Yong-Hee Kim**[1], Seung-Jung Ha[1], Ki-Jung Kim[1], Bang-Jin Kim[1], Byung-Gak Kim[1], Mi-Sun Jung[1], In-Cheul Kim[2], Sun-Ho Choi[2], Buom-Yong Ryu[1]

[1]Animal Science and Technology, Chung-Ang University, Ansung, Korea, Republic of, [2]National Institute of Animal Science, Cheonan, Korea, Republic of

In livestock, important potential of techniques including isolation and cultivation of spermatogonial stem cells (SSCs) has been emphasized to study for producing transgenic animal and conserving excellent or rare genetic resource because the cells are unique adult stem cells to contribute genes to the next generation. Although long-term culture techniques were not available for porcine SSCs, cryopreserved testis cells or tissue from boars provides opportunity to long-term preservation and enhancements of SSCs availability. Thus, the aim of this study was to find an efficient cryopreservation method for porcine SSCs.

Obtained testes from 16-week-old pig were cut into pieces of small fragments, approximately 1 x 1 x 2 mm^3 after removing the tunica albuginea, rete testis and overt connective tissue. Approximately 1 gram of testis tissues (tissue freezing group) or testicular cells recovered by 2 steps enzyme treatments (cell freezing group) were suspended in freezing media consisting of MEM alpha with 10% dimethyl sulfoxide (DMSO) and 10% FBS without additional supplements (basal freezing medium) or basal freezing medium with the addition of 200 mM trehalose in 1.8 ml cryovials. The vials were placed in a Nalgene freezing container, and frozen at -80°C for overnight, after which the vials were plunged into liquid nitrogen for 30 days. After thawing, at 37°C water bath for 2.5 minutes, both groups were cultured for 10 days to determine proliferation capacity of germ cells freeze-thawed; In case of tissue freezing group, testicular cells were recovered by 2 steps-enzyme treatments after thawing. Proliferating spermatogonial cells after culture were determined by the number of germ cells expressing protein gene product 9.5 (PGP 9.5) per a gram of tissue freeze-thawed, significantly greater improvement was obtained in tissue freezing with trehalose (17.4 ± 2.0) compared to control (without treatment of trehalose, 3.0 ± 0.8) and others. SSC xenotransplantation of testis cell populations from tissue freezing with trehalose group generated significantly more colonies (78.6 ± 4.75) of donor derived germ cells than control (27.5 ± 2.1). Collectively, these results demonstrate that tissue freezing with treatment of 200 mM trehalose is prospective cryopreservation method for porcine SSCs and this method may contribute to research about preservation of livestock SSCs.

This research was supported by the Next-Generation BioGreen 21 Program (No.PJ009554), the Korean Health Technology R&D Project (No.A120080), Ministry of Health & Welfare and Rural Development Administration, the National Research Foundation of Korea (NRF) grant funded by the Korean Government (MEST) (20120006786).

F-2423

EFFECTS OF SUGAR MOLECULES ON SURVIVAL AND FUNCTIONAL CAPACITY IN CRYOPRESERVATION OF MURINE SPERMATOGONIAL STEM CELLS

Yong-An Lee, Ki-Jung Kim, Yong-Hee Kim, Bang-Jin Kim, Byung-Gak Kim, Mi-Sun Jung, Seung-Jung Ha, Buom-Yong Ryu

Chung-Ang University, Ansung, Korea, Republic of

On account of characteristics that spermatogonial stem cells (SSCs) are essential to maintaining male fertility and species continuity, cryopreservation of SSCs has potential value for related study, like the transgenic animal production and male infertility. The effects of sugar molecules on the cryopreservation of mammal cells have been well known. In order to improving survival of SSCs during cryopreservation process, the influence of sugar molecules on the cryopreservation of SSCs were investigated in the present study.

Testis cells were collected from a pup transgenic mouse line C57BL/6-Tg (EGFP) and purified via magnetic activated cell sorting (MACS) technique with anti-Thy-1 antibody after enzymatic digestion using 0.25% trypsin and 7 mg/mL DNase I. Subsequently, they were cultured under serum-free conditions consisted with mitotically inactivated STO cell feeders and growth factors including 10 ng/mL GDNF, 75 ng/mL GFRα1 and 1 ng/mL bFGF. 4 weeks after germ cell culture initiation, cultured germ cells were isolated by digestion in 0.25% trypsin and suspended at 5 X 10^5 cells/mL in basal freezing medium (MEM alpha with 10% dimethylsulfoxide and 10% FBS) as a control or in basal freez-

ing medium with addition of 50 mM, 100 mM or 200 mM various sugar molecules in cryovials. The cryovials were frozen by conventional slow-freezing protocol and kept in liquid nitrogen for 3 months. Frozen cells were thawed by rapid thawing and evaluated for proliferation potential. Although we found the dose-dependent increase in treatment groups of fructose, mannose, sucrose, maltose and trehalose, proliferation potential was significantly greater for germ cells frozen in fructose (152.6 ± 11.2%), mannose (158.7 ± 10.8%) and trehalose (171.7 ± 10.4%) at a concentration of 200 mM compared to control (100 ± 0%) and all other groups. In addition, transplantation analyses showed that normal colonies were generated from donor SSCs freeze-thawed with fructose, mannose or trehalose. The results indicated that sugar molecules including fructose, mannose, sucrose, maltose and trehalose are effective cryoprotectant. Additionally, fructose, mannose and trehalose at a concentration of 200 mM are most suitable sugar molecule among others as additives to freezing medium for SSCs.
This research was supported by the Next-Generation BioGreen 21 Program (No.PJ009554), the Korean Health Technology R&D Project (No.A120080), Ministry of Health & Welfare and Rural Development Administration, the National Research Foundation of Korea (NRF) grant funded by the Korean Government (MEST) (20120006786).

F-2424

IDENTIFICATION OF DIFFERENT POPULATIONS OF SPERMATOGONIAL STEM CELLS IN NEWBORN AND ADULT MARMOSETS

Daniel Langenstroth[1], Nina Kossack[1], Jörg Gromoll[1], Rüdiger Behr[2], Stefan Schlatt[1]
[1]Centre of Reproductive Medicine and Andrology, Münster, Germany, [2]Stem Cell Biology Unit, German Primate Center, Göttingen, Germany

Introduction:
Marmoset monkeys (Callithrix jacchus) are highly similar to humans in terms of their testicular development and are therefore widely used as a non-human primate model in preclinical reproductive research. In the adult testis, spermatogonial stem cells (SSCs) represent the most undifferentiated germ cells. These cells can self-renew and differentiate into sperm throughout male life. However, in contrast to the germ cells that are present in the newborn testis, adult SSCs seem to have a more restricted differentiation potential, as they do not express any pluripotency markers such as OCT3/4. In this study we aim to elucidate whether germ cells from the newborn and the adult marmoset can be enriched and maintained in culture and whether they differ with regard to their differentiation status.
Materials and Methods:
Testes were obtained from newborn (n = 5) and adult (n = 6) marmoset monkeys from the institutional breeding colony. To establish cell cultures marmoset testes were enzymatically digested, plated onto culture dishes and cultured in MEMα + 10 % FBS + 1 % Pen/Strep. After 3, 6 and 11 days the cells from the supernatant (SN) were separated from the attached cells (AT). Subsequently, qPCR analyses were performed using marker genes for pluripotency and undifferentiated germ cells (OCT3/4, LIN28), differentiating germ cells (VASA, MAGE-A4) as well as somatic cells (VIM, αSMA). In addition, cultures were analyzed on days 6 and 11 using immunofluorescence (IF) stainings.
Results:
During the first 3 days of adult marmoset testicular cell culture about 7 % of the cells attached to the culture dish. The remaining SN cells partially formed clusters of up to 30 cells. However, from day 3 to day 11 the total cell number in the SN decreased from 4.7 to 2.4 million, whereas it increased in the AT fraction from 0.3 to 0.6 million. qPCR and IF analyses revealed that germ cells expressing VASA and MAGE-A4 were enriched in the SN fraction and could be maintained for the culture period of 11 days. Moreover, the cell clusters in the SN exclusively expressed VASA and MAGE-A4 but no somatic markers. Conversely, the AT fraction was enriched for somatic cells expressing αSMA and VIM.
Strikingly different, the SN obtained from the newborn marmoset testes did not form clusters and marker gene expression revealed drastically declining expression levels of pluripotency marker genes OCT3/4 and LIN28 and the germ cell marker gene VASA. This indicates that germ cells from the newborn marmoset could not be maintained for more than 3 days using the same conditions as for the adult testicular cells.

Conclusion:
In this study, we demonstrate for the first time, that germ cells from the adult marmoset monkey can be enriched via the separation of the SN from the AT fraction and that these cells can be maintained during short-term culture. However, low expression levels of OCT3/4 and LIN28 indicate a low multi-lineage differentiation potential of these cells.
Based on the finding that the required conditions for culturing SSCs from newborn and adult testes differ, we intend to explore the characteristics of the different populations in more detail. We conclude that different types of SSCs exist in the testes of primates which may be associated with specific developmental stages. This may have important implications for understanding male infertility and stem cell damage in man.

F-2431

ISOLATION OF SPERMATOGONIAL STEM CELLS FROM OCT4-EGFP TRANSGENIC PIGS

Monika Nowak-Imialek[1], Nico Lachmann[2], Doris Herrmann[1], Heiner Niemann[1]
[1]Biotechnology, Friedrich-Loeffler-Institut, Neustadt, Germany, [2]REBIRTH Research Group Reprogramming, Hannover Medical School, Hannover, Germany

We have produced the first germ line transgenic pigs carrying the entire 18 kb genomic sequence of the murine Oct4 gene fused to the enhanced green fluorescent protein (EGFP) cDNA (OG2 construct) (Nowak-Imialek et al., 2011, Stem Cells and Development). Expression of the EGFP reporter construct is confined to germ line cells, the inner cell mass and trophectoderm of blastocysts, and testicular germ cells, incl. putative spermatogonial stem cells (SSCs). SSCs are unique among stem cells because they can both self-renewal and differentiate into spermatozoa. In-depth knowledge on SSCs in pigs has been hampered by the inability to isolate these cells from the complex cell population of the testis. In the Oct4-EGFP transgenic mouse, SSCs are the only adult stem cells that express Oct4. Here, we used Oct4-EGFP transgenic pigs as model to isolate and characterize Oct4-EGFP cells in the adult porcine testes. Fluorescence microscopy of testicular tissue isolated from transgenic piglets revealed a very low number of EGFP positive cells, whereas testicular tissue isolated from adult transgenic boars contained a high amount of EGFP fluorescent cells. Northern blot analysis confirmed stronger EGFP expression in the testis of adult transgenic pigs than in the testis from transgenic piglets. The time course and the signal intensity of EGFP expression in Oct4-EGFP testis paralleled mRNA expression of the endogenous Oct4 gene. SSCs can be identified using germ cell specific surface markers by flow cytometry and immunohistochemistry. We report the identification of surface antigenic characteristics (THY1, CD49f, CD29, SSEA-4) in porcine testis successfully used in other species for enrichment of SSCs. For derivation of SSCs, the testes were enzymatically dissociated using two digestion steps to obtain a single-cell suspension. Flow cytometry revealed distinct populations of THY1 (14%), CD49f (68%), CD29 (24%) and SSEA-4 (5%) positive cells in the adult porcine testes. Thereafter fluorescence-activated cell sorting (FACS) based on Oct4-EGFP and additionally THY1 expression was successfully used to purify specific testicular cell populations. Four cell populations, i.e. EGFP+/THY1-, EGFP+/THY1+, EGFP-/THY1+ and EGFP+/THY1+ could be isolated. Real-time PCR analysis revealed expression of germ cell specific markers in both EGFP+ (14%) and THY1+ (4.7%) cell populations. Further characterization of THY1 and EGFP expressed cells in the porcine testis sections using immunohistochemistry is currently underway.

F-2432

TESTICULAR VERY SMALL EMBRYONIC-LIKE STEM CELLS (VSELS) RESIST CHEMOTHERAPY AND UNDERGO SPERMATOGENESIS WHEN A HEALTHY 'NICHE' SUPPORT IS PROVIDED

Deepa Bhartiya, Sandhya Anand, Kalpana Sriraman
Stem Cell Biology, National Institute for Research in Reproductive Health, Mumbai, India

Various adult body organs including gut, skin, bone marrow etc harbor quiescent and actively dividing stem cell populations. Our group has reported the presence of relatively quiescent VSELs in adult mammalian testis along with actively dividing 'progenitors' A_{dark} spermatogonial stem cells (SSCs). A_{dark} SSCs exist as chains due to incom-

plete cytokinesis and further differentiate and undergo meiosis to form the haploid sperm. The present study was undertaken to study the effect of oncotherapy on testicular VSELs. Further since the testicular microenvironment 'niche' is compromised due to oncotherapy, we investigated whether transplanting healthy niche cells can stimulate the VSELs to undergo spermatogenesis.
Testicular tissue of busulphan treated mice and azoospermic survivors of childhood cancer showed the presence of VSELs in otherwise germ cell depleted tissue based on histology, immunolocalization and presence of pluripotent (Oct-4A, Nanog, Sox-2), primordial germ cell (Stella) and VSEL specific (CD133 in humans and Sca-1^+ Lin^- $CD45^-$ in mouse) markers. Inter-tubular random injections, into the germ cell depleted busulphan treated mouse testis, of syngenic Sertoli cells or bone marrow derived mesenchymal cells were able to restore spermatogenesis from persisting VSELs, as evident by altered histology, presence of sperm in caudal epididymis and q-PCR analysis. Transplanted Sertoli or mesenchymal cells possibly were a source of growth factors/ cytokines essential for VSELs differentiation. Since sperm formation occurred *in situ*, various epigenetic concerns associated with 'synthetic gametes' are eliminated in our approach. This basic study provides an exciting alternative to restore fertility in cancer survivors.

F-2433

IDENTIFICATION OF PHOSPHORYLATED TIF1β EXPRESSED IN GENITAL TISSUES OF ADULT MICE

ying Y. wang
National Institute of Advanced Industrial Science and Technology(AIST) Research Center for Stem Cell, ibaraki, Japan

Transcription networks composed of various transcriptional factors specifically expressed in undifferentiated embryonic stem (ES) cells have been implicated in the regulation of pluripotency in ES cells. However, the molecular mechanisms responsible for self-renewal, maintenance of pluripotency, and lineage specification during differentiation of ES cells are still not fully understood.
We have recently found that a phosphorylation-dependent chromatin relaxation factor, transcriptional intermediary factor-1β (TIF1β), is a unique regulator of the pluripotency of ES cells and regulates Oct3/4-dependent transcription in a phosphorylation-dependent manner.
TIF1β is specifically phosphorylated in pluripotent mouse ES cells at the C-terminal serine 824, which has been previously shown to induce chromatin relaxation. Phosphorylated TIF1β induces ES cell-specific genes and enables prolonged maintenance of an undifferentiated state in mouse ES cells. Moreover, TIF1β regulates the reprogramming process of somatic fibroblasts to iPS cells in a phosphorylation-dependent manner.

Pre-clinical and Clinical Applications of Mesenchymal

F-3001

INTRACEREBROVENTRICULAR TRANSPLANTATION OF HUMAN MESENCHYMAL STEM CELLS IMPROVES THREE CORE AUTISTIC-LIKE BEHAVIOURAL PHENOTYPES OF BTBR MICE

Daniel Offen[1], Golan Karvat[2], Hadar Segal[1], Ran Barzilay[1], Javier Ganz[1], Liat Edri[2], Tali Kimchi[2]
[1]Tel Aviv Univ, Peta-Tikva, Israel, [2]Department of Neurobiology, Weizmann Institute of Science Israel, Rehovot, Israel

Background: Autism spectrum disorders (ASD) are neurodevelopmental disorders characterized by social interaction impairment, communication deficits and repetitive stereotypic behaviors. BTBR inbred mice are a commonly used model of ASD as they demonstrate robust behavioral deficits consistent with the three diagnostic criteria for ASD. Moreover, BTBR mice also exhibit decreased BDNF signaling and reduced hippocampal neurogenesis. Current available therapeutics offer no cure for ASD patients. Mesenchymal stem cells (MSC) possess great potential for regenerative therapy in brain diseases. Thus in this study, we aimed to investigate the possible improvement in the autistic-like behavioral phenotypes in BTBR mice after transplantation of human MSC.
Results: Transplantation of 105 human MSC cells into the lateral ventricles of 6-8 week-old BTBR mice resulted in a reduction of the stereotypical grooming and digging behaviors, elevation of social behavior and a decrease in cog-

nitive rigidity, as observed by the jammed running wheel test, three-chamber assay and the "wet" T-maze. Importantly, analysis of the overall standardized autism score revealed highly significant differences between MSC- and saline-treated subjects. A month after transplantation, some human MSC survived in the mice brain throughout the course of the experiment, as revealed by immunohistochemical assay. Using ELISA, we also found increased BDNF levels in both the hippocampus and the frontal cortex in the MSC-treated mice. Remarkably, a significant positive correlation between BDNF levels and behavioral improvement of autistic-like behavioural phenotypes was detected.
Conclusions: Delivery of MSC to lateral ventricles is beneficial in attenuating behavioral deficits manifested in the BTBR mouse model of ASD. The increased BDNF levels which correlate with the observed amelioration in autistic-like behavior might indicate the possible mechanism underlying this improvement. Our study suggests a novel therapeutic approach for treating ASD animal models which may be translatable to ASD patients.

F-3002

AXON REGENERATION AFTER PLURIPOTENT MESENCHYMAL STEM CELL TRANSPLANTATION IN CHRONIC INTRACEREBRAL HEMORRHAGE MODEL

Yao LIU, Jing Ye ZHOU, Ling Fei LI, Kam Sze Kent TSANG, Gang* LU, Wai Sang POON
Division of Neurosurgery, Department of Surgery, The Chinese Univ of Hong Kong, Shatin, Hong Kong

Although cell therapy is increasingly indicated to be efficient in many neurodegenerative animal models, the positive result in clinical application was poor. In this study, we aimed to investigate the mechanism of axon degeneration and regeneration after intra-ventricular transplantation of a kind of pluripotent mesenchymal stem cell in our modified chronic intracerebral hemorrhage (cICH) mouse model. CD45 and TER119 negative eGFP cells were isolated by micro-magnetic beads from bone marrow mononuclear cells, then cultured in DMEM/F-12 with EGF, PDGF-BB and LIF. 10ul of 2.5E5 cells were injected to the lateral ventricle of cICH murine models. The neurological improvement was assessed by using behavioral tests including Cylinder tests, Water Maze and Gait analysis. Then the axon outgrowth was demonstrated by using anti-NF staining in both pre-hematoma brain and bilateral pontile fibers, which was further confirmed by quantitative PCR and western blotting of the axon-remodeling markers including MBP, NF-M and GAP-43. Data suggested that in the first weeks after transplantation, the gait parameters were with no significant differences in treatment group compared with saline-treat group. After 2 weeks post-grafting, the several parameters in Gait analysis (including LF print area and Stance) were different in two groups. Histology analysis showed an increasing expression of NF-positive fibers in pre-hematoma tissue and additional qPCR analysis confirmed that an increasing expression of MBP, NF-M and GAP-43 in pontile fibers in MSCs-treated group. It is indicated that we have provide a detailed analysis of axon regeneration events in the MSCs transplanted ICH mice, and the conduction possibilities of these regenerated fibers will be further investigated. Works were supported by HongKong RGC Grant (473709) and CUHK Direct Grant (2041730).

F-3003

MSC LICENSING BY BIOMECHANICAL FORCE

Siobahn M. Evans[1], Daniel J. Kota[2], Robert A. Hetz[2], Scott D. Olson[2], Fabio Triolo[2], Charles S. Cox, Jr.[1], Pamela L. Wenzel[1]
[A]Center for Stem Cell and Regenerative Medicine, Institute of Molecular Medicine; Pediatric Surgery, [B]Pediatric Surgery, [1]University of Texas Medical School at Houston, Houston, TX, United States

The beneficial effects of Mesenchymal Stromal Cell (MSC)-based cellular therapies are mediated primarily by the ability of the MSC to suppress inflammation associated with chronic or acute injury, graft-versus-host disease, and autoimmunity. MSCs express extremely low levels of immunogenic cell surface molecules and, as a result, the engrafted cells are less prone to rejection and can be collected from non-human leukocyte antigen (HLA)-matched donors. Indeed, the broad potential for treatment of patients with a wide array of conditions warrants a more thor-

ough understanding of the effectors of MSC immunomodulatory function and how these anti-inflammatory signaling pathways are activated.
MSCs are known to require licensing of their immune-modulatory function by exposure to inflammatory cytokines and/or direct interaction with activated immune cells. Naive MSCs do not express key mediators of immunosuppression, such as the multifunctional anti-inflammatory protein TNF-α stimulated protein 6 (TSG-6), prostaglandin E_2 (PGE_2), and interleukin (IL)-1 receptor antagonist (IL1RN). Here, we report a critical role for an unexpected environmental cue, fluid shear stress, as a determinant of expression of these and other anti-oxidative factors that contribute substantially to MSC immune regulation.
MSCs derived from three human tissue sources, bone marrow, adipose, and amniotic fluid, were all found to be responsive to shear stress to varying extents. In evaluation of bone marrow-derived MSCs, we found that laminar shear stress typical of hemodynamic flow in major arteries of the adult can stimulate profound up-regulation in transcription of *TSG-6*, *COX-2*, *IL1RN*, *HMOX-1*, *LIF,* and *KLF2,* ranging from 6- to 120-fold increases. Importantly, changes in expression of these anti-inflammatory and anti-oxidative factors lead to a functional enhancement of MSCs to inhibit production of cytokines involved in systemic inflammation, such as tumor necrosis factor-alpha (TNF-α), that are released by activated (M1) macrophages and CD4+ T lymphocytes. Naive MSCs exposed to shear stress, with no preconditioning by inflammatory cytokines, were capable of blocking TNF-α secretion by lipopolysaccharide (LPS)-activated mouse splenocytes (ranging from complete inhibition to 2-fold reduction below MSC cultured under static conditions, depending upon MSC donor and source variability). Ongoing analyses with models of traumatic brain injury will reveal whether these observed effects translate to *in vivo* enhancement in function.
In summary, MSCs are a promising and abundant source of adult stem cells that can abrogate the damaging effects of unchecked systemic inflammation. By studying the role of biomechanical forces in MSC immune regulation, we have uncovered a novel method for licensing MSCs that promises to provide immediate anti-inflammatory protection to the patient, even prior to onset of inflammatory response. Improved understanding of signaling pathways downstream of mechanical cues will be critical to development of more efficacious cellular
therapies and is expected to translate to greater treatment options for conditions such as neural injury, stroke, myocardial infarction, autoimmune disease, and autoinflammatory disorders.

F-3004

EFFECTS OF EXOSSOMES PROVIDED FROM BONE MARROW MESENCHYMAL STEM CELLS IN RATS TREATED WITH LIPOPOLYSACCHARIDE.

Luciana A. Reis, Fernanda T. Borges, Joelma S. Christo, Manuel J. Simões, Nestor Schor
Nephrology Division-Medicine Dept, Escola Paulista de Medicina/UNIFESP, Sao Paulo, Brazil

Introduction: Sepsis is characterized by a severe inflammatory response to infection, and its complications, including acute kidney injury (AKI). Studies indicate that the possible mechanism of BMSCs actions may be due to paracrine modulation, releasing biological factors such as microvesicles (MVs) type exosomes (EXOs). The aim of this study is to evaluate the EXOs effects on AKI induced by E.coli lipopolysaccharide (LPS) in rats. Methods: Bone marrow mesenchymal stem cells (BMSCs) were incubed with DMEM without SBF during 12 hours. Then, the conditioned medium (CM) were collected and EXOs were obtained by ultracentrifugation technique and characterized by transmission electron microscopy. Groups include (n=10 each): LPS (10mg/Kg/BW, IV, 3 times); PBS (CTL); LPS+ EXOs (100µg/ml, IV, 3 times). Blood were collected for creatinine (Cr, mg/dl) and urea (U, mg/dl) measurements and the animals were sacrificed 72 hr. after treatments. Kidneys were perfused and removed for HE, KI67 and caspase 3 analyses. Results: EXOS were able to substantially minimize ($p<0.05$) the impact of LPS on renal function, as seen by creatinine (0.8±0.1 vs. 2.3±0.1) and urea (69 ±3 vs. 149.7±6) concentrations and are similar ($p>0.05$) with CTL group (0.5±0.1 and 38±3, for Cr and U, respectively). In LPS-group, the kidneys showed a small marked KI67 and intensive caspase 3 expression but differently, it was highly marked for KI67 and lower expression for caspase 3 in LPS+EXOs groups and no histological acute tubular necrosis lesions were observed. Although not show, comparing 1 with 3 EXOs applications, these effects were enhanced when evaluated by higher KI67 and lower caspase 3. Conclusion: These results strongly suggest that EXOs derived from BMSCs can minimize AKI in this sepsis model. These therapeutics EXOs effects have a significant impact on renal function and holds substantial potential use

especially by avoiding transplanting cells with potential adverse effects, in this experimental model. Funds from: FAPESP, CNPq, CAPES and FOR.

F-3005

MESENCHYMAL STEM CELL-BASED THERAPY USING STRONTIUM INCORPORATED HYDROXAPATITE FOR OSTEOPOROTIC BONE RECONSTRUCTION.

Sunitha Chandran, Suresh Babu S., Hari Krishnan V.S., H.k. Varma, Annie John
Division of Implant biology (TEM Laboratory), Sree Chitra Tirunal Institute For Medical Sciences And Technology, Thiruvananthapuram, Kerala., India

Osteoporosis and resorptive pathologies are on the rise worldwide leading to a large population with fracture prone skeletal tissue. Mesenchymal stem cells (MSCs) that mediate bone healing have slower growth rate and reduced osteogenic potential resulting in delayed fracture healing in Osteoporotic patients. Due to enhanced bone resorption than formation, osteoporotic fracture fixation often leads to implant loosening and failure. An ideal strategy would address the resorption, prevent further bone loss and stimulate new bone formation thus strengthening the bone-implant interface.
Taking into account, the potential application of strontium (Sr) in osteoporosis, attributed to its dual role in osteogenesis and resorption, the influence of Sr on the osteogenic differentiation of MSC has important implications. Strontium incorporated Hydroxyapatite (HA.Sr) micro-granules has been indigenously developed into a functional unit with MSCs. This is in view of the fact that the cellular part would facilitate in osteogenesis and Sr ion would serve as an anti-resorbing agent, thus accomplishing a bi-functional single implant unit. Hence transplanting osteogenically differentiated bone marrow cells seeded on HA.Sr would improve bone defect healing.
HA (control) and HA.Sr were characterized by Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM) for its functional groups, size (350-400 microns) and 3 D porous surface topography. Further, dissolution study of HA.Sr indicated greater efficiency to induce apatite formation, a characteristic feature that favor osteogenesis.
To fabricate tissue-engineered constructs, rat bone marrow-derived Mesenchymal Stem Cells (rBMSCs) were isolated, characterized by flow-cytometry, osteogenically induced and then seeded (1 x 10^5 cells/cm2) on HA.Sr (cHA.Sr) and HA (cHA). Cell adhesion, cyto-compatibility and viability were assessed by SEM, fluorescent staining (DAPI-Rhodamine staining) and MTT assay. Furthermore osteogenically induced cHA.Sr depicted improved ALP expression (ELF - 97) under Confocal microscopy. Simultaneously, Alizarin and von Kossa staining was depicted which indicated that Sr accelerated Calcium deposition and mineral apatite, a criteria again favoring osteogenesis.
As proof of concept, the efficacy of cHA.Sr substitute to heal critical-sized femur ‘drill hole’ defects (1.5mm deep and 3mm long) was evaluated in rat osteoporotic model, developed by ovariectomy and evaluated by Micro CT. Six weeks post implanted bone samples were assessed by micro CT and histology of stevenal’s blue van gieson’s picrofuschin stained plastic sections. Interestingly, cHA.Sr substitute depicted faster bone regeneration in par with material degradation compared to cHA implants.
Cell-based tissue engineering in conjunction with strontium incorporated Hydroxyapatite enhanced bone repair via osteoinduction, osteoconduction and osteointegration. This becomes applicable especially in osteoporotic clinical situations of trauma where in the MSC niche of the host itself is impaired and bone bonding is an essential criteria to strengthen bone-implant interface in fracture repair.

F-3006

HUMAN MESENCHYMAL STROMAL CELLS MAY LEAD TO OPPOSITE THERAPEUTIC RESULTS AGAINST 4T1 MURINE BREAST TUMOR MODEL IN DIFFERENT APPROACHES

Tatiana Jazedje[1], Mayra Pelatti[2], Heloisa M. de Siqueira Bueno[2], Elaine G. Rodrigues[1], Mayana Zatz[2]
[1]Federal University of Sao Paulo, Sao Paulo, Brazil, [2]University of Sao Paulo, Sao Paulo, Brazil

The use of Mesenchymal Stromal Cells (MSCs) aiming to treat cancer is very contradictory. Several protocols have been used, with very discrepant results, in particular, because there are many types of tumors, animal models and sources of human and animal MSCs. Although several studies report the worsening of symptoms after co-injection of MSCs with tumor cells in animal models, some reports show clinical improvement when MSCs were injected intravenously or intraperitoneally in animals that already had tumor growth. Our group described, for the first time, the presence of MSCs in the human tube (human tube Mesenchymal Stromal Cells- htMSCs). Since breast cancer affects mainly women, in an attempt to understand the possible role of MSCs against cancer, the aim of this study was to evaluate the therapeutic effect of htMSCs in the treatment of murine mammary adenocarcinoma using two different approaches: (1) Co-injection of MSCs and 4T1 tumor cell lineage. For this approach, we used 12 eight weeks-old immunocompetent mice Balb-c females, divided into 2 groups: G1- co-injected with 10e6 htMSCs and 10e4 4T1 and G2- untreated control group, with cancer (injected with 10e4 4T1). (2) Injection of htMSCs in animals that were in the initial stage of the disease. For this, 18 eight-week old immunocompetent mice Balb-c females were injected with 10e4 4T1 cells and animals were divided into 3 groups: G3- treated with 1 injection of htMSCs 7 days after the 10e4 4T1 cells injection, G4- treated with 2 injections of htMSCs 7 and 14 days after the 10e4 4T1 cells injection, and G5- untreated control group, only injected with 10e4 4T1 cells. In all groups injections were made into the mammary adipose tissue. Animals were evaluated up to 20 days and were periodically assessed. In the first experiment all animals developed primary tumor locally with or without htMSCs injection. Co-injected animals (G1) survived only 15 days and the necropsies showed many tumor masses in the abdominal region and lungs, much worse than the untreated group (G2), that presented only primary tumor growth and no visible nodules in the abdominal region and lungs. In experiment 2, animals that received 2 injections of MSCs (G4) presented primary tumors 20% smaller, on average, than the control group (G5). Necropsies were analyzed 20 days after 4T1 cells injection in the 3 groups. No or just few abdominal nodules were found in some animals of the 3 groups, but no lung metastatic focus were clearly evident in any group, which might be due to the short experimental time. The role of MSCs in animals with cancer is unclear. Our observations suggest, for the first time, that the same MSCs to treat the same kind of murine mammary adenocarcinoma, in the same animal model, can lead to opposite results, worsening or improving the outcome, depending on the experimental procedure. A possible hypothesis to explain these observations is that MSCs would enhance the invasor effect of tumor cells when they share the same microenvironment, but they can be beneficial in animals with tumors already established. In order to confirm these observations, further experiments with larger groups are currently underway.

F-3007

TARGETING INSULIN RESISTANCE IN TYPE 2 DIABETES THROUGH IMMUNE MODULATION OF CORD BLOOD-DERIVED MULTIPOTENT STEM CELLS IN STEM CELL EDUCATOR THERAPY: PHASE I/II CLINICAL TRIAL

Yong Zhao[1], Zhaoshun Jiang[2], Zhaohui Yin[2], Heng Li[3]

[1]University of Illinois at Chicago, Chicago, IL, United States, [2]General Hospital of Jinan Military Command, Jinan, China, [3]Shandong University, Jinan, China

The prevalence of type 2 diabetes (T2D) is increasing worldwide and creating a significant burden on health systems, highlighting the need for the development of innovative therapeutic approaches to treat this disease. Mounting evidence points to the involvement of immune dysfunction in insulin resistance in T2D, suggesting that immune modulation may be a useful tool in treating T2D. Previous works demonstrated that Stem Cell Educator therapy provides lasting reversal of immune dysfunctions and allows regeneration of islet β cells and improvement of metabolic control in patients with long-standing type 1 diabetes. This groundbreaking study has been highlighted by the American Diabetes Associated at 72nd Scientific Sessions (ADA, Philadelphia, 2012) as one of eight major breakthroughs and initiatives in 2012. Here, we explore the therapeutic potential of Stem Cell Educator therapy in T2D subjects.

Objective: To explore the safety and efficacy of Stem Cell Educator therapy in overcoming insulin resistance through the immune modulation of cord blood-derived multipotent stem cells (CB-SCs) in patients with long-standing type 2 diabetes (T2D).

Design, Setting, and Patients: In an open-label, phase1/phase 2 study, patients (N = 36) with long-standing T2D were divided into three groups (Group A, oral medications; Group B, oral medications + insulin injections; Group C having impaired β-cell function with oral medications + insulin injections). Median age was 51 years (median diabetic duration, 9 years).
Intervention: All subjects received one treatment with the Stem Cell Educator therapy in which a patient's blood is circulated through a closed-loop system that separates mononuclear cells from the whole blood, briefly co-cultures them with adherent CB-SCs for 8 - 9 hours in vitro, and returns the educated autologous cells to the patient's circulation.
Main Outcome Measures: Changes in glycated hemoglobin (HbA1C) values and islet β-cell function of T2D between baseline and follow-up.
Results: Clinical findings indicate that T2D patients achieve improved metabolic control and reduced inflammation markers after receiving Stem Cell Educator therapy. Median HbA_1C in Group A and B was significantly reduced from 8.61% ± 1.12 at baseline to 7.9% ± 1.22 at 4 weeks (p = 0.026), 7.25% ± 0.58 at 12 weeks (p = 2.62E-06), and 7.33% ± 1.02 at one year post treatment (p = 0.0002). Homeostasis model assessment (HOMA) of insulin resistance (HOMA-IR) demonstrated that insulin sensitivity was improved post treatment. Notably, the islet β-cell function in Group C subjects was markedly recovered, as demonstrated by the restoration of C-peptide levels. Mechanistic studies revealed that Stem Cell Educator therapy reverses immune dysfunctions through immune modulation on monocytes and balancing Th1/Th2/Th3 cytokine production.
Conclusions: This study demonstrates that Stem Cell Educator therapy can control the immune dysfunctions and restore the immune balance through the modulation of monocytes, leading to a significant improvement of insulin sensitivity and metabolic control in long-standing moderate or severe T2D subjects. This novel approach holds great promise for improving treatment and finding a cure for diabetes. The advantages of Stem Cell Educator therapy may help diabetics to cope with diabetes-associated complications and improve their life quality, without have the safety and ethical concerns associated with conventional stem cell-based approaches.

F-3008

HUMAN DENTAL PULP STEM CELLS INTERACT WITH THE BLOOD BAIN BARRIER

Simon A. Koblar[1], Joshua Winderlich[2], Karlea Kremer[2]
[1]Univ of Adelaide, Adelaide, Australia, [B]Medicine, [1]Univ of Adelaide, Adelaide, Australia

Cell-based therapy in ischaemic stroke is an important new strategy to enhance functional outcome following brain damage. Our group has recently published data that adult stem cells derived from the dental pulp of third molar teeth (Dental Pulp Stem Cells; DPSC) from young humans when injected into the rodent brain 24 hours post-stroke enhanced functional recovery. However, this method of delivery resulted in a 9% mortality in this rodent model which would be unacceptable in a clinical situation. There are a number of reported studies that demonstrate the vascular delivery of stem cells post-stroke may also provide similar functional benefit. We asked the question if adult human DPSC interact with the blood-brain-barrier (BBB) in a way which may allow the entry of stem cells into the brain. In order to study this interaction we developed an in vitro model of the BBB using a transwell millipore system growing rat-derived astrocytes on one side and human endothelial cells on the other. We treated this in vitro BBB with media from DPSC cultures and found that this resulted in an increase in permeability of the BBB demonstrated by diffusion of various dyes and that occludin protein expression was down-regulated. This appeared to be mediated by a non-VEGF protein. These results indicate stem cells are able to interact with the BBB in a manner which would increase their access to the brain in ischaemic stroke.

F-3012

EPHRINB2 INVOLVED IN REGENERATION OF THE BM VASCULATURE NICHE AFTER MYELOSUPPRESSION

Yan Sun, Kimberly Canty, Leslie Silberstein
Children's Hospital Boston, Boston, MA, United States

HSCs interact with multiple cell types within the BM microenvironment and have been shown to reside in close association with bone marrow (BM) osteoblasts in the endosteal niche and also in proximity to BM sinusoidal vessels. Recent findings show vascular reconstitution of the bone marrow is important to hematopoietic regeneration after myelosuppression with cytotoxic agents or whole-body irradiation. However, the phenotypic signature and molecular pathways involved in regeneration of the BM vasculature need to be further defined. Ephrin-B2,a transmembrane ligand for Eph receptors, possesses intrinsic signalling capabilities that are required for early angiogenic remodeling. Our hypothesis is whether EphB2 contributes to the process of blood vessel regeneration after myelosuppression damages the bone marrow (BM) vascular niche. Here we identified that, under steady-state conditions, EphB2 is not expressed in HSPCs, but is expressed in multipotent PDGFR+and Sca-1+ mesenchymal stem cell (PαS) populations. Further, we found that EphB2 expression robustly increases in PαS cells post-irradiation (650 rad and 950 rad), and returns to normal level in these primitive cells during vascular niche recovery. However, expression is unchanged in the HSPC population. Accordingly, the immunofluorescent staining results from early stage post-irradiation revealed no co-localized expression of EphB2 and laminin, a blood vessel marker, in blood vessels. However, during late stages of recovery, blood vessels show high expression of EphB2. The change in EphB2 expression in the PαS cell population during the transition between myelosuppression and recovery is due to the EphB2+ cells, which differentiate to endothelial cells, contributing to vessel regeneration. The normal EphB2 expression in the PαS cells is reestablished. Overall, these results provide preliminary evidence for EphB2 as a contributor to blood vessel regeneration and remodeling in the BM vascular niche after myelosuppression damage.

F-3013

ISOLATION OF A NOVEL POPULATION OF MESENCHYMAL STEM CELLS FROM FETAL HEART AND ITS THERAPEUTIC EFFICACY IN MYOCARDIAL INFARCTION

Soniya Nityanand[1], Garikipati VN Srikanth[1], Sachin Jadhav[1], Naresh K. Tripathy[1], Deepa Kapoor[2]
[A]Hematology, [B]Gynaecology Unit, General Hospital, [1]Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India

Mesenchymal stem cells derived from fetal heart (fC-MSC) may be particularly suitable for cardiac regenerative therapy because of their tissue of origin. We have isolated fC-MSC from hearts of 5 human fetuses of 12 weeks gestational age. The fC-MSC exhibited extensive expansion potential without senescence and with maintenance of a normal karyotype over successive passages. The fC-MSC constitutively expressed cardiovascular markers c-kit, Isl-1, Flk-1, Nkx2.5, GATA-4 and MDR-1 and could be differentiated into all the three major cell types of the cardiovascular lineage (cardiomyocytes, endothelial cells and smooth muscle cells). Furthermore, the fC-MSC expressed embryonal markers Oct-4, Nanog, Sox-2, TRA-1-60 and TRA-1-81 and in addition to mesodermal differentiation, they could also be differentiated into ectodermal (neuronal) and endodermal (hepatocytic) lineages.
The therapeutic efficacy of fC-MSC was evaluated in a rat left anterior descending coronary artery ligation model of myocardial infarction (MI). fC-MSC obtained from rat fetuses at gestation day 16 were labeled with PKH26 and injected through tail vein on day 7 of MI induction and results compared with rats in which saline was injected (n=6 in both the groups). The rats were sacrificed 4 weeks after therapy and heart tissues evaluated for (i) presence of PKH-26 labelled fC-MSC by immunohistochemistry; (ii) co-localization of immunostaining for cardiac Troponin T (cTnT), smooth muscle cell myosin heavy chain (sm-MHC) and CD31 with PKH26 labelling; (iii) expression of growth factor- and anti-apoptotic genes using Real time PCR; and iv) myocardial fibrosis and infarct size by Masson's Trichome staining and Triphenyl Tetrazolium Chloride (TTC) staining, respectively. Further, the non-perfused area of infarcted myocardium and the left ventricle (LV) function were monitored in both the groups of rats by serial 99Tc-sestamibi pinhole gated SPECT-CT performed before and up to 4 weeks after therapy.
PKH-26 labelled fC-MSC could be detected in the infarct/peri-infarct region 4 weeks after therapy and co-expressed cTnT, CD3 and sm-MHC indicating ability of fC-MSC to differentiate into cardiomyocytes, endothelial and smooth muscle cells, respectively. fC-MSC treated hearts showed significant up regulation in expression of growth factors VEGF, βFGF, IGF-1, HGF-1 and TGF-β, compared to saline treated group and a significant decrease in TUNEL positive cells in comparison to saline treated rats ($p<0.05$). Masson's Trichome staining demonstrated attenuation of the myocardial fibrosis and TTC staining showed a significant reduction in the infarct size in the fC-MSC treated in comparison to the saline treated group of rats ($p<0.05$). The fC-MSC treated group showed a significant increase in ejection fraction (EF), and a significant decrease in end diastolic volume, end

systolic volume and left ventricular (LV) myomass compared to saline treated group ($p<0.05$). In addition, perfusion studies demonstrated smaller ischemic lesion and a significantly greater 99mTc-sestamibi uptake in hearts treated with fC-MSC in comparison to saline treated group ($p<0.05$). Our data shows that fC-MSC are novel multipotent MSC with large scale expansion potential and cardiovascular pre-commitment and fC-MSC therapy in rats with MI leads to attenuation of the infarct size and functional improvement of the cardiac functions, indicating a potential therapeutic role of these stem cells in cardiac regenerative medicine.

F-3014

AUTOLOGOUS TRANSPLANTATION OF GDNF-EXPRESSING MESENCHYMAL STEM CELLS PROTECTS AGAINST MPTP-INDUCED DAMAGE IN CYNOMOLGUS MONKEYS

Zhenhua Ren, Jiayin Wang, Shuyan Wang, Chunlin Zou, Xiaobo Li, Yuanqian Guan, **Zhiguo Chen**, Yu Alex Zhang
Xuanwu Hospital Capital Medical Univ, Beijing, China

Glial cell-derived neurotrophic factor (GDNF) has shown beneficial effects in rodent and primate models of Parkinson's disease (PD). However, the mild results observed in a double blind clinical trial by intraputamenal infusion of recombinant GDNF warrant a search for alternative delivery method. In this study, we investigated the function of autologous mesenchymal stem cells (MSCs) expressing GDNF (GDNF-MSCs) for protection against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced injury in cynomolgus monkeys. MSCs were obtained from the bone marrow of individual monkeys and gene-modified to express GDNF. Following unilateral engraftment of GDNF-MSCs into the striatum and substantia nigra, the animals were challenged with MPTP to induce a stable systemic Parkinsonian state. The motor functions were spared in the contralateral limbs of monkeys receiving GDNF-MSCs, but not in those receiving MSCs alone. In the striatum of the grafted hemisphere, the dopamine levels were higher and the dopamine uptake was also enhanced as indicated by Single Photon Emission Computed Tomography (SPECT). The results suggest that autologous MSCs may be a safe vehicle to deliver GDNF for enhancing the nigro-striatum functions in PD patients.

F-3015

HUMAN MSCS PRODUCING HUMAN G-CSF, A THERAPEUTIC CANDIDATE FOR ISCHEMIC STROKE.

M G Muralidhar[1], Chiara Leroy[1], Antti Nurmi[2], Nina Vartiainen[2], Alex Kharazi[1]
[1]R & D, Stemedica Cell Technologies, San Diego, CA, United States, [2]Charles River Discovery Research Services, Kuopio, Finland

Stroke is one of the leading causes of death and a major disease burden for humanity with a paucity of effective therapies against it. Human Mesenchymal Stem Cell (hMSC) treatments have shown to result in reduction of disability in animal models of stroke.
G-CSF protein has anti-apoptotic and neuroprotective properties and it is a promising therapeutic candidate for stroke. G-CSF is not normally secreted by hMSCs, but we have been able to engineer these cells to produce human G-CSF. Given the therapeutic potential of both, hMSCs and G-CSF, this study was aimed at evaluating the efficacy of G-CSF producing hMSCs compared to non-producing hMSCs in a rodent model of cerebral ischemia.
hMSCs were isolated from bone marrow aspirates and expanded in culture. Adenoviral vector containing human G-CSF sequence was constructed by standard recombinant DNA technologies and viral particles generated in HEK 293 cells. hMSCs were transduced by exposing them to vector particles, harvested and stored in liquid nitrogen. Cell marker analyses by flow cytometry, ability to differentiate or production of VEGF did not differ significantly between G-CSF transduced and non-transduced cells. However, transduced cells secreted robust amounts of human G-CSF (over 1ug per million cells were secreted over 24 hours) and secretion was maintained over several days in vitro.
In vivo properties of the G-CSF transduced vs. non-transduced cells were studied by evaluating indium (111In) labeled cells and their biodistribution with SPECT imaging and gamma counting in rats subjected to transient middle cerebral artery occlusion (tMCAO). Results revealed that the distribution of these cells in vivo was very sim-

ilar between G-CSF transduced and non-transduced cells, with initial accumulation in lungs followed by significant shift in activity to liver, spleen and kidneys. Highest observed signal in the brain was at 24h after the injection but it was only about 1% from the maximum activity observed in the lungs at 1h post injection.
Finally, we studied the efficacy of G-CSF transduced and non-transduced hMSCs against experimental stroke in rats. Twenty-four hours after tMCAO, cells were injected IV and functional deficits in rats was monitored over the next 14 days. Based on preliminary behavioral assessments with 7-point neuroscore and amphetamine induced rotation asymmetry test, rats treated with G-CSF transduced cells recovered better if compared to non-transduced or vehicle treated group. Further experiments are currently ongoing to verify the positive therapeutic effect of G-CSF transfected cells in rodent models of stroke.
To summarize, these results suggest that engineered G-CSF expressing hMSCs show similar in vitro characteristics as non-transduced hMSCs. Distribution of G-CSF transduced cells in vivo was also found to be almost identical to non-transduced hMSCs. Interestingly, there was no increase in signal of G-CSF transduced cells in the brain after stroke, although preliminary experiments showed better functional recovery compared to non-transduced hMSCs. Further experiments are warranted to elucidate the possible therapeutic properties of G-CSF producing hMSCs in stroke and other neurological disease models.

F-3016

NOVEL ISOLATION AND EXPANSION TECHNIQUE TO ENHANCE THE REGENERATIVE POTENTIAL OF HUMAN BONE MARROW STEM CELLS

Jae-Suk Jung, Jong-Bin Lee, In-Kyeong Lee, Eun-Young Choi, Soyon Park, Yerin Hong, Kyoo-Sung Cho, Chang-Sung Kim
Department of Periodontology, Research Institute for Periodontal Regeneration, College of Dentistry, Yonsei University, Seoul, Korea, Republic of

Objectives: Many studies have shown that hypoxic culture condition helps the stem cells to preserve their self renewal potential and to enhance tissue regeneration capacity. The aim of this study was to demonstrate the novel isolation technique using highly cross-linked hydronate filter (CHF) for the human bone marrow stem cells (hBMSCs) and the expansion under hyaluronate-mediated hypoxic condition (EHC) for the enhancement of tissue regeneration.
Materials and Methods: In the present study, human bone marrow was harvested from the vertebral body during vertebral surgery. CHFs were used for isolation of the mononuclear cell (MNC) layer from human bone marrow. MNC layers conjugated with CHFs were transplanted directly into the ectopic transplantation model *in vivo* and seeded into culture dishes and incubated to allow the attachment of adherent cells. Single-cell colonies were observed and passage (P0) cells were cultured under the hyaluronate-mediated hypoxia condition for expansion, and then characterized using a colony-forming unit(CFU) assay, proliferation, migration, *in vitro* differentiation, and *in vivo* ectopic transplantation assay. Throughout the study, cells isolated using conventional gravity gradient isolation technique and expansion under normoxia condition were used for control. Cells at P3-P5 were used for the study.
Results: BMSCs of novel isolation technique using CHF and expansion under hyaluronate-mediated hypoxic condition potentially possess superior qualities to that of conventional isolation technique. Direct transplantation of MNC layers conjugated with CHFs successfully generated new bone, cartilage and bone marrow tissue in the ectopic transplantation model. The hyaluronate-mediated hypoxic condition applied to hBMSC during expansion enhanced the CFU efficiency of these cells, increased their proliferation and migration potential, and reduced their differentiation potential *in vitro*. However, the ectopic transplantation of these hBMSC with hyaluronic acid as a cell carrier *in vivo* increased the formation of bone and bone marrow like tissue .
Conclusion: The novel use of CHF for hBMSC isolation from the human bone marrow followed by hyaluronate-mediated hypoxic culture technique can be a good and effective alternative for clinical application and for the enhancement of regeneration potential of hBMSC.
Acknowledgement: This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MEST) (No. 2012M3A9C6049862)

F-3017

NOVEL APPLICATION OF HUMAN BONE MARROW STEM CELLS WITH FGF-2 FOR COLLAGEN TISSUE REGENERATION

In-Kyeong Lee, Jong-Bin Lee, Jae-Suk Jung, Eun-Young Choi, Soyon Park, Yerin Hong, Kyoo-Sung Cho, Chang-Sung Kim

Department of Periodontology, Research Institute for Periodontal Regeneration, College of Dentistry, Yonsei University, Seoul, Korea, Republic of

Objectives: Mesenchymal stem cells (MSCs) have been introduced as alternative filling materials in the field of esthetic and reconstructive soft tissue augmentation due to their ability to produce substantial amount of collagen, a main soft tissue component. The aim of this study was to assess the effects of fibroblast growth factor-2 (FGF-2) on collagen tissue regeneration by human bone marrow stem cells (hBMSCs) in *in vitro* and *in vivo.*

Materials and Methods: hBMSCs were isolated from bone marrow of the body of human vertebra during a vertebral surgery. To confirm the stem cell population in the isolated cell group, a series of *in vitro* and *in vivo* analyses were performed including colony forming unit (CFU) assay, cell-surface-marker characterization, *in vitro* multidifferential potency, self-renewal potency and *in vivo* ectopic soft tissue regeneration using mouse model. The cell proliferation and cell migration of hBMSCs in various concentrations of FGF-2 (0, 0.5, 1.25, 5, 12.5 ng/ml) were analyzed. Insoluble/soluble collagen syntheses were also assessed by evaluating collagen related markers including lysyl oxidase (LOX), lysyl oxidase like (LOXL)1, LOXL2, and hydroxyproline. *In vivo* collagen formation was examined after hBMSCs were transplanted using hyaluronic acid as a cell carrier into the subcutaneous pockets of immunocompromised mice. Histologic and immunohistochemistry analyses were performed 8 weeks later (n = 4 at each concentration of FGF-2).

Results: hBMSC population with the characteristics of mesenchymal stem cells was present in isolated cells of the vertebra. There was a FGF-2-dose-dependent enhancement in hBMSC proliferation and cell migration. The amount of insoluble/soluble collagen formed was significantly enhanced in the FGF-2 treated group than the untreated control group ($p < 0.05$). The histologic and immunohistochemistry results revealed that the amount of collagen formed *in vivo* was greater and more structuralized in the FGF-2 treated group at 8 weeks ($p< 0.05$) although a dose dependent pattern was not shown above 1.25ng/ml.

Conclusion: FGF-2 facilitates the properties of hBMSCs, rendering them more suitable for cosmetic soft-tissue augmentation.

Acknowledgement: This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MEST) (No. 2012M3A9C6049862)

F-3018

HUMAN PLACENTA-DERIVED MULTIPOTENT CELLS MODULATE CARDIAC INJURY THROUGH BOTH CARDIOMYOGENESIS AND PRONAGIOGENESIS -- FROM BENCH TO LARGE ANIMAL STUDY

Yuan-Hung Liu[1], Kai-Yen Peng[2], Yu-Wei Chiu[1], Yi-Lwun Ho[3], Shih-Yun Huang[1], Yi-Shuan Lin[1], Betty Linju Yen[4], Men-Luh Yen[2]

[1]Section of Cardiology, Cardiovascular Center, Far Eastern Memorial Hospital, New Taipei City, Taiwan, [2]Department of Primary Care Medicine and Department of Obstetrics/Gynecology, National Taiwan University, Taipei, Taiwan, [3]Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan, [4]Institute of Cellular & System Medicine, Division of Regenerative Medicine, National Health Research Institutes, Taipei, Taiwan

Recent studies have shown that human placenta-derived multipotent cells (hPDMCs) are capable of multilineage differentiation. The lack of ethical concerns in procurement of these multilineage progenitors and their immunomodulatory properties make them good candidates for cell therapy of damaged organs. However, the feasibility of hPDMCs in cardiac repair is unclear.

We found that the cardiomyogenic gene expression of hPDMCs is much higher than other human somatic cells. Upon co-culture with mouse neonatal cardiomyocytes, hPDMCs were documented to differentiate into striated cardiomyocytes. Transplantation of hPDMCs into the hearts of severe combined immunodeficiency mice after LAD artery ligation significantly improved left ventricular function, with significantly enhanced vascularity in the cell-treated group. The proangiogenic effects were further confirmed *in vitro* by tube-formation assays using human endothelial cells. These proangiogenic effects were mediated by hPDMC secretion of HGF, GRO-α, and IL-8. The minipigs undergoing hPDMCs treatment after myocardial infarction showed significant improvement of contractility than the control group ($p=0.016$) at 8 weeks post injury. Tissue analysis confirmed the cardiomyogenesis differentiation of hPDMCs *in vivo*.
Our findings offer solid evidence that hPDMCs can modulate cardiac injury in both small and large animal model, possibly through both cardiomyogenesis and proangiogenesis. This study offers some mechanistic insights and preclinical evidence on using hPDMCs as a therapeutic strategy to treat severe cardiovascular diseases.

F-3021

HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS REPAIR THE ARTICULAR CARTILAGE DESTRUCTION IN OSTEOARTHRITIS MICE MODEL

Yu Hsun Chang[1], Dah-Ching Ding[2]
[1]Department of Pediatrics, Tzu Chi General Hospital, Hualien City, Taiwan, [2]Institute of Medical Sciences, Tzu Chi University, Hualien City, Taiwan

Osteoarthritis (OA) is a chronic degenerative joint disorder which is
characterized by articular cartilage destruction and osteophyte formation. Tissue engineering and stem cell therapy have been applied in the
osteoarthritis research which can provide a permanent, biological solution. Usually, the OA animal model used larger animal such as rat or guinea pig. We successfully established mice OA model.
We used a single intraarticular injection of 0.1 mg monosodium
iodoacetate (MIA) through the infrapatellar ligament of the bilateral knees of NOD-SCID mice. We found chondrocytes loss which present in typical pathological changes in OA. We also performed functional exercise study by RotaRod 3376-4R (TSE systems, Chesterfield, MO, USA).
Our human umbilical cord mesenchymal stem cells (HUCMSCs) were
positive for CD34, CD45, CD73, and HLA-DR, but positive for CD44, CD90 and HLA-A, -B, -C. In chondrogenic medium, the HUCMSCs had the capability of chondrogenesis. In MIA-induced NOD/SCID OA murine model, four weeks after HUCMSCs transplantation, the HUCMSCs treated mice got significant improvement of joint function (one week after MIA treatment, MIA group vs. HUCMSCs group: 34% vs. 37% of original function, $p=0.574$; 28 days without/with HUCMSCs transplantation, MIA group vs. HUCMSCs group: 20% vs. 111% of original function, $p<0.001$) and histological improvements demonstrated by Hematoxylin and eosin stain.
In conclusion, HUCMSCs transplantation effectively repaired the
osteoarthritis both in functional and histological aspects.

F-3022

MODULATION OF MESENCHYMAL STEM CELL IDO ACTIVITY THROUGH INTRACELLULAR MICROPARTICLES

James A. Ankrum[1], Ong Joon Faii[1], Riddhi G. Dastidar[1], Oren Levy[1], Debanjan Sarkar[2], Jeffrey M. Karp[1]
[1]Department of Medicine, Center for Regenerative Therapeutics, Brigham and Women's Hospital, Harvard Medical School, Harvard-MIT Division of Health Sciences & Technology, Harvard Stem Cell Institute, Cambridge, MA, United States, [2]Department of Biomedical Engineering, University at Buffalo, The State University of New York, Cambridge, MA, United States

Statement of Purpose: Mesenchymal stem cells (MSCs) are of great interest for the treatment of a multitude of inflammatory conditions including graft versus host disease, Crohn's disease, and acute lung injury. While MSCs are being investigated in over 200 clinical trials for their immunomodulatory properties, MSCs fail to persist long term in vivo limiting their therapeutic effect. Therefore, there is a great need to augment MSC therapy. We address this problem by enhancing MSCs' immunomodulatory phenotype. We have previously developed a method to control MSC fate through internalized drug loaded microparticles. Herein we use this technique to enhance the immunomodulatory potential of MSCs.
Methods: As steroids have previously been shown to increase the expression of indoleamine 2,3-dioxygenase (IDO) in astrocytes, we sought to investigate the effect the steroid budesonide (BUD) has on MSC IDO activity. After treating MSCs with 1 μM BUD for 24 hours, MSCs were additionally exposed to 100ng/ml IFN-γ for 48 hours. MSCs were collected, lysed to isolate IDO from the cells, and the quantity of L-kynurenine produced within 1 hour was measured with a colorimetric assay. The upregulation of IDO upon BUD exposure was further confirmed by western blot analysis. BUD-PLGA particles were formed through a single emulsion encapsulation technique and the size was adjusted through alteration of the agitation speed. DLS and HPLC were used to measure the particles size and drug loading, respectively. MSCs were loaded with 0.1 mg/mL BUD-PLGA particle suspensions, with or without poly-L-lysine, in 1% serum media and incubated overnight. To characterize particle internalization, flow cytometry and confocal microscopy were performed. L-kynurenine production from BUD-particle modified MSC was measured to assess the ability of BUD-Particle to enhance IDO activity.
Results: Treatment of MSCs with BUD resulted in significantly increased IDO activity ($p<0.001$) . MSCs preferentially internalized 1 μm BUD-PLGA particles compared to 1.4 μm particles, and internalized positively charged particles over negatively charged particles. Particle loading of MSCs resulted in internalization of 15-20 particles/cell as shown by confocal analysis. BUD-Particle modified MSC exhibited a 4-fold increase in IDO activity compared to unmodified and Blank-Particle modified MSCs ($p<0.001$).
Conclusions: BUD-Particle modification enhanced MSC immunomodulatory potential via a 4X increase in IDO activity. To our knowledge, this is the first report of drug induced enhanced IDO activity in MSCs. MSC readily internalized 1 μm BUD-PLGA particles and while the number of particles internalized per cell is heterogeneous, most cells in the population internalized particles. Thus, this platform should be useful to significantly enhance the immunomodulatory properties of MSCs post transplantation to suppress T-cell proliferation, induce T-regulatory cells, and polarize macrophages to an M2 phenotype.

F-3024

MESENCHYMAL STEM CELL (MSC) THERAPY TO IMPROVE LIMB TRANSPLANT OUTCOME

Shashikumar Salgar[1], Emilie Fitzpatrick[2], Mary DeHart[1], Juan Tercero[1], Tommy Brown[2]
[A]Clinical Investigation, [B]Surgery, [1]Madigan Army Medical Center, Tacoma, WA, United States

Introduction: Hand and leg transplantation (Tx) in military and civilian populations is increasingly needed to improve the quality of life of individuals. Optimizing nerve regeneration and preventing immune rejection are vital to successful outcomes in limb transplantation. In this study we investigated whether MSC therapy can improve nerve regeneration and function in rat limb transplant model.

Methods: Orthotopic syngeneic right hind-limb transplants were performed in Lewis (RT1.A^l) rats. The donor and recipient femoral artery/vein were anastamosed by vascular cuff method, and the sciatic nerve (epineurium) and muscles were approximated by suturing. The femur was stabilized with a stainless steel pin and bone cement. Following transplantation animals received MSC (5×10^6; passage ≤7) or vehicle (saline control) locally at the surgical site, and 5×10^6 MSC intravenously on the day of surgery. MSC or vehicle injections (i.v.) were repeated every week for four weeks. Walking track analysis and cutaneous pain reaction tests were performed at 1-2 week intervals post-Tx.

Results: Rat MSC expanded *ex vivo* were $CD29^+$, $CD90^+$, $CD34^-$, $CD31^-$, $CD45^{low}$, MHC Class I^+, Class II^-, $CD80^{low}$, and $CD86^-$. MSC were pluripotent and differentiated in to adipocytes, osteocytes and chondrocytes in *ex vivo* cultures under specific conditions. At 4 weeks post-Tx, sensory nerve function as determined by cutaneous pain reaction

test was <0.6 on a scale of Grade 0-3 (0=No function; 3= Normal function). However, by 8 weeks in vehicle treated animals (n=7) it was 2.2±0.7 (tibial), 1.1±0.5 (peroneal) and 1.5±0.9 (sural), and in MSC treated animals (n=6) it was 2.6±0.5 (tibial), 1.9±0.5 (peroneal) and 1.9±0.7 (sural). The sensory nerve function was significantly ($p<0.05$) higher at 8 and 12 weeks compared to 4 weeks post-Tx. Interestingly, peroneal ($p<0.05$), tibial ($p>0.05$) and sural ($p>0.05$) nerve functions were higher in stem cell treated group compared to vehicle treated group. Walking track analysis did not produce clear foot prints to calculate Sciatic Function Index (SFI). Laser doppler analysis revealed normal vascularization and limb transplant survival was >90%.

Conclusions: The limb transplant procedure was highly successful, and MSC therapy appears to promote nerve function recovery. The study is ongoing and awaiting data on long-term effects of MSC on limb function.

F-3025

A THERAPEUTIC STUDY BY TRANSPLANTATION OF AMNIOTIC FLUID STEM CELLS COULD RESCUE THE LIVER CIRRHOSIS MODEL IN MICE

Steven Shaw[1], SHAO-YU PENG[2], PO-JEN CHENG[1], WINSTON TK CHENG[3], Shinn-Chin WU[3]

[1]Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, Taoyuan, Taiwan, [A]INSTITUTE OF BIOTECHNOLOGY, [B]Department of Animal Science and Technology, [2]NATIONAL TAIWAN UNIVERSITY, TAIPEI, Taiwan

Introduction

Amniotic fluid stem cells (AFSCs) can be expanded without feeder layers and can differentiate into mesenchymal lineages. The liver cirrhosis model could be created by regular injection of carbon tetrachloride (CCl_4) dissolving in the sunflower oil. We aimed to explore the therapeutic effect and stem cell fate after transplantation of AFSCs in this liver injury model of mice.

Methods

The wild type mice received intravenous injection of CCl_4 (2ml/kg) twice per week from 8 week-old. The experimental animals were divided into three groups: untreated group, sham group, and CCl_4 treated group (n=20, per group). One million Ds-red harboring porcine amniotic fluid derived stem cells (Ds-red pAFSCs) was injected into the mice from 12 week-old to rescue the liver poisoning. The body weight and liver function including glutamic-oxalocetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), and albumin were measured before the liver injury and after stem cell therapy. Hydroxyproline is the indicator for the severity of liver fiber and collagen injury was also measured.

Results

The liver cirrhosis model in mice was successful created after injection on CCl_4 at 12 week-old. The CCl_4 treated group showed the significant higher level of GOT, GPT, albumin, shorter survival and lighter body weight compared to untreated or sham group ($p<005$). After Ds-red pAFSCs xenotransplantation, the stem cell therapy demonstrated the dramatic decreasing of all liver function level in CCl_4 treated mice. The mice were sacrificed at 16 week-old. The liver sections of Ds-red pAFSCs treated mice were found less fibrotic scars confirmed by Masson's Trichrome staining and less hydroxyproline level compared to other groups. Immunostaining further presented the red fluorescence single could be detected clearly in the mice hepatocytes.

Conclusion

This is the first therapeutic study by using amniotic fluid stem cells to rescue the classic liver cirrhosis model in mice. We have demonstrated xenotransplantation of Ds-red pAFSCs into the injured mice could improve the liver function, body weight, better survival and less liver fibrotic scaring. The AFSCs could be the cell therapy source to treat the liver disease in the future.

F-3026

EFFECTS OF SMALL MOLECULES (LOSARTAN AND VITAMIN C) ON CELL THERAPY MEDIATED REGENERATION IN MUSCLE INJURED MICE

Eun-Mi Lee, Ah-Young Kim, Eun-Joo Lee, Chang-Woo Min, Kyung-Ku Kang, Myeong-Mi Lee, Meeyul Hwang, Sang-Hyeob Kim, Soo-Eun Sung, Kyu-Shik Jeong
College of Veterinary Medicine, Kyungpook National University, Daegu, Korea, Republic of

Skeletal muscle has high regenerative potential, but has low cellular turnover. That is why stem cell therapy is required in some of case, including volumetric muscle loss or severe hereditary disorders such as Duchenne muscular dystrophy (DMD). Additionally, it is well known that it is essential to make a favourable stem cell niche to induce the proper settlement of the transplanted stem cells. In this study, we investigated about how some small molecules (losartan and vitamin C), which are known as materials that improve the stem cell niche, will affect transplanted stem cells.
First, we studied whether combined treatment the adipose-derived stem cells (ASCs) with losartan in muscle injured-mdx mice enhance muscle regeneration. In gross and microscopic findings, ASC transplanted mice with treatment of losartan showed enhanced muscle regeneration compared to the mice were not. In additions, treatment of losartan decreased the serum transforming growth factor-β (TGF-β) and down-regulated mRNA of collagen type I. As a results, reduced muscular fibrosis was observed in ASCs and losartan combined treated group.
Second, we studied whether vitamin C improves the niche for stem cell transplantation based on its potent antioxidant effects. In both gross and microscopic observations, mice treated with vitamin C exhibited more complete regeneration of injured muscles than those of vitamin C depleted mice. In addition, reduced carbonylated protein groups, which are the end-products of oxidative stress, were detected in vitamin C-supplied groups compared in vitamin C-depleted groups. The difference is clearer in the presence of transplanted stem cells. Moreover, the serum total vitamin C level and ascorbic acid (AA) to dehydroascorbic acid (DHA) ratio also were decreased in the presence of transplanted ASCs. Taken together, these data can be considered as proof of vitamin C utilization by cells in vivo. Meanwhile, the vitamin C-depleted groups showed more weak fibrosis than that of the vitamin C-supplied groups.We demonstrasted that effects of losartan and vitamin C on stem cell therapy for muscle regeneration. Consequently, our results will help choice of small molecules to use for improvement of stem cell therapy efficiency.

F-3027

PROFILING OF THE SECRETOME OF MESENCHYMAL CELLS FROM DIFFERENT SOURCES

Reaz Vawda, Alejandro Jose, Anna Maria Badner, Michael George Fehlings
University Health Network, Toronto, ON, Canada

Background : Spinal cord injury (SCI) is a debilitating condition without cure that affects more than 1.5million people in the US alone, costing the healthcare system $10billion annually. The high cost reflects the predominance of cervical injuries amongst sufferers, who are likely to experience varying spinal cord level-dependent degrees of severity of sensorimotor and autonomic deficits. The latter often worsen with time. SCI is hence described as consisting of the primary injury and a subsequent progressive cascade of events known as the secondary injury. Cell therapy is particularly well suited to addressing the multi-factorial and dynamic nature of the pathophysiology of secondary SCI. Rationale : Mesenchymal or stromal cells are currently in several clinical trials for SCI, reflecting the recognition of their vast therapeutic potential. However, a limited understanding of their biology impedes a full-scale clinical deployment. Aims : We have undertaken to characterise the secretome of mesenchymal cells from a range of sources in order to identify similarities and differences between them. This would (1) enable the optimisation of the source and culture conditions for maximal therapeutic benefit in in vivo models of SCI, and (2) help elucidate their mechanisms of action. Methods : The relative concentrations of factors in media conditioned by cells were examined using ELISA arrays. Results : Umbilical cord matrix cells demonstrated higher expression of several molecules with known roles in angiogenesis (activin, angiogenin, angiopoietin-1, amphiregulin and coagulation factor III), inflammation (G-CSF, GM-CSF, IL-6, IL-8, ENA-78, MCP-3, midkine, MIP-1alpha/beta, and MIP-3alpha), and neural cell survival, proliferation and differentiation (FGF2, FGF7 and GDNF). The latter factors were not found in adult BMSC conditioned media. Conclusions : Our preliminary results indicate that umbilical cord matrix secretome is likely to have greater therapeutic potency than those from other cell types and sources. Future work : Through a systematic comparison of the effects of different cell sources and types cultured under specific

conditions, we will identify putative molecular mediators of in vitro effects on neural progenitor cell survival, proliferation and differentiation and in vivo effects on several parameters of secondary SCI models.

F-3028

MESENCHYMAL PRECURSOR CELLS REDUCE HISTOPATHOLOGICAL DISEASE PROGRESSION OF COLLAGEN INDUCED ARTHRITIS IN AN OVINE MODEL OF RHEUMATOID ARTHRITIS

Anwar M. Abdalmula[1], Simon R. Bailey[1], Elizabeth A. Washington[1], Laura Dooley[1], Jackie House[2], Peter Ghosh[3], Silviu Itescu[3], Wayne G. Kimpton[1]

[1]Veterinary Science, University of Melbourne, Melbourne, Australia, [2]Florey Institute of Neuroscience and Mental Health, Melbourne, Australia, [3]Mesoblast Ltd, Victoria, Australia

Rheumatoid Arthritis (RA) is an autoimmune disease driven by Th17 T cells, a pro-inflammatory monocyte phenotype, activated synoviocytes, and erosive arthritis. We evaluated whether allogeneic ovine immunoselected and culture-expanded Mesenchymal Precursor Cells (MPCs) could inhibit Th17 and monocyte activation, and reduce erosive joint pathology in a collagen induced arthritis (CIA) model in sheep that shares many pathological and immunological features of human RA. CIA was established in thirty six merino sheep. Two weeks after an intra-articular collagen injection into the left hock joint and established arthritis, sheep were randomized to receive a single intravenous injection of either 0.3, 1 or 2 million allogeneic MPCs/kg or saline, and followed for 30 days before euthanasia. In saline treated control sheep, histopathological analysis of hock joints demonstrated that the left hock synovium was characterized by leukocyte infiltration, synovial hyperplasia, pro-inflammatory cytokine expression, and cartilage erosion. In comparison with saline treated controls, synovial tissue from arthritic sheep receiving a single intravenous injection of 2 million MPCs/kg showed 88% mean reduction in cellular IL-6 levels (p=0.029), 83% mean reduction in cellular TNF-alpha levels (p=0.049), 53% mean reduction in cellular IL-17 levels (p=0.005), and 52% mean reduction in infiltrating monocytes/macrophages (p=0.009). MPC-treated animals had a 31% mean reduction in histopathology severity scores compared with controls (p=0.025). These results indicate that a single IV injection of MPCs in an ovine model of collagen-induced RA attenuates joint inflammation and cartilage erosion. Together, these data suggest that MPCs may represent a first line treatment to attenuate joint inflammation and RA disease progression by dual inhibition of the Th17 T cell subset and monocyte-derived production of multiple pro-inflammatory cytokines.

F-3031

A NOVEL ANIMAL COMPONENT FREE CULTURE SYSTEM FOR ISOLATION AND EXPANSION OF HUMAN BONE MARROW DERIVED MESENCHYMAL CELLS

Ravenska Wagey[1], Jacky Yau[1], Matthew Wong[1], **Erik B. Hadley**[1], Chris Duronio[1], Bari Zahedi[1], Cindy Miller[1], Terry E. Thomas[1], Allen C. Eaves[2], Sharon A. Louis[1]

[1]STEMCELL Technologies Inc., Vancouver, BC, Canada, [2]Terry Fox Laboratory, Vancouver, BC, Canada

Human mesenchymal progenitor cells (MPCs) are a heterogeneous cell population typically isolated from bone marrow (BM) aspirates and cultured in the presence of animal-derived serum. When MPCs are to be used in cell therapy applications the presence of xenogeneic serum poses safety concerns such as animal to human viral transmission. The aim of this study was to develop a fully-defined, serum-free and animal component-free (ACF) medium (MesenCult™-ACF) that enables efficient isolation, enumeration and expansion of MPCs from primary human BM. Primary human BM was processed by Ficoll® density separation and the mononuclear cells set up in 1) colony-forming unit fibroblast (CFU-F) assays and 2) expansion cultures. Three different media were compared: MesenCult™-ACF (ACF), MesenCult™-Xeno Free (XF) and serum-containing medium. The plastic ware for culturing cells in ACF and XF media was pre-coated with attachment substrate. Clonogenic growth of MPCs was evaluated in CFU-F assays by plating BM mononuclear cells in multiple wells at low seeding densities (10,000 - 50,000 cells/cm^2)

in each medium. In expansion cultures, primary BM cells were initially plated at a range of 7 x 10^4 - to 1.3 x 10^5 cells/cm^2 in separate wells using the 3 media. For subsequent subculture, cells were seeded at 1500 - 3000 cells/cm^2 and the proliferative potential of MPCs in each medium was determined by counting the total cell number obtained at each serial passage up to Passage 8 (P8). Cells expanded in the three media were also characterized at P3 and P6 for their multi-lineage potential. The presence of adipocytes were determined by Oil red O staining, osteogenic cells by Alizarin Red staining and chondrocytes by Alcian Blue and/or Collagen staining. Total number of CFU-F (per 1 x 10^6 mononuclear cells) was 60 ± 11 in ACF, 57 ± 7 in XF and 39 ± 7 in serum-containing media (mean ± SEM; n=4). Average cell expansion of cultured MPCs from P1 to P8 in ACF was higher (5.0 ± 0.3 fold; mean ± SEM; n=5) compared to serum-containing medium (3.6 ± 0.4 fold; n=6) at each subculture. Average cell expansion in XF medium was 9.6 ± 1.0 fold (n=7). Importantly, cells cultured in defined ACF medium maintained the capacity to differentiate into adipocytes, osteogenic cells and chondrocytes. In addition, when transitioned into the new MesenCult™-ACF medium, cells previously cultured in serum-containing medium or XF medium could be efficiently expanded without the need for cell transitioning procedures. Further studies to more fully characterize MPCs cultured in MesenCult™-ACF are underway and will be reported. In summary, the data indicate that our novel MesenCult™-ACF media is able to support attachment and enrichment of MPCs, produce efficient clonogenic growth and long-term cultures directly from primary human BM under animal component-free culture conditions. This is the first fully-defined ACF culture system which produces high quality MPCs with no exposure to serum or xenogeneic components from cell sample processing to *ex vivo* cell expansion, thus meeting a critical need in the field of translational research.

F-3032

MICRORNA 455 TARGETING SOCS3 PROMOTES JAK-STAT SIGNALING FOR CELL SURVIVAL IN HYPOXIC RAT MESENCHYMAL STEM CELLS

Se-Yeon Lee[1], Byeong-Wook Song[1], Min-Ji Cha[1], Onju Ham[1], Chang Youn Lee[2], Jun-Hee Park[2], Jiyun Lee[1], Hyang-Hee Seo[1], Minji Seung[1], Eunhyun Choi[3], Eunmi Choi[4], Il-Kwon Kim[5], **Ki-Chul Hwang**[4]
[1]Yonsei University College of Medicine, Brain Korea 21 Project for Medical Science, Seoul, Korea, Republic of, [2]Graduate School, Yonsei University, Department of Integrated Omics for Biomedical Sciences, Seoul, Korea, Republic of, [3]Yonsei University Health System, Severance Integrative Research Institute for Cerebral & Cardiovascular Disease, Seoul, Korea, Republic of, [4]Yonsei University College of Medicine, Severance Biomedical Science Institute, Seoul, Korea, Republic of, [5]Yonsei University College of Medicine, Cardiovascular Research Institute, Seoul, Korea, Republic of

Mesenchymal stem cells (MSCs) are an attractive candidate to repair damaged cardiac tissue and improve left ventricular function. However, many studies showed that MSCs may not be survive well and harsh environment of the infarcted heart induces MSC apoptosis. Therefore, protection strategy of MSCs against apoptosis is significant for successful cellular therapy. MicroRNAs (miRs) are lately considered that involved in development, differentiation, metabolism and apoptosis. Here, we hypothesized that miR-455 would enhance the survival of rat MSCs against induction of apoptosis in hypoxic condition. We selected 14 miRs which may have anti-apoptotic functions and transfected with all miRs mimics to confirm the survival rate by using cell counting and MTT assay. Among them, miR-455 is the most meaningful miR which could enhance survival of MSCs in hypoxic condition. We predicted a specific target of miR-455, suppressor of cytokine signaling-3(SOCS3) which induces apoptosis by negative regulation of JAK-STAT pathway. The protein level and luciferase activity of SOCS3 were decreased in miR-455-transfected MSCs. The proteins of JAK-STAT signaling, phosphorylated-PI3K, -AKT, and -mTOR, were also highly regulated in miR-455-overexpressed MSCs. Taken together, these data indicate that miR-455 is a critical miRNA to promote survival of MSCs in hypoxic condition by targeting SOCS3.

F-3033

MICRORNA 146A MODULATES ANGIOGENIC EFFECTS OF HUMAN SYNOVIAL FLUID-DERIVED MESENCHYMAL STEM CELL TARGETING NEUROFIBROMIN 2

Hyang-Hee Seo[1], Byeong-Wook Song[1], Onju Ham[1], Se-Yeon Lee[1], Chang Youn Lee[2], Jun-Hee Park[2], Jiyun Lee[1], Minji Seung[1], Eunhyun Choi[3], Eunmi Choi[4], Il-Kwon Kim[5], **Ki-Chul Hwang**[4], Woochul Chang[6]
[1]Yonsei University College of Medicine, Brain Korea 21 Project for Medical Science, Seoul, Korea, Republic of, [2]Graduate School, Yonsei University, Department of Integrated Omics for Biomedical Sciences, Seoul, Korea, Republic of, [3]Yonsei University Health System, Severance Integrative Research Institute for Cerebral & Cardiovascular Disease, Seoul, Korea, Republic of, [4]Yonsei University College of Medicine, Severance Biomedical Science Institute, Seoul, Korea, Republic of, [5]Yonsei University College of Medicine, Cardiovascular Research Institute, Seoul, Korea, Republic of, [6]The Catholic University of Korea College of Medicine, Institute of Catholic Integrative Medicine, Seoul, Korea, Republic of

Mesenchymal stem cells (MSCs) can be extracted from adipose, peripheral blood, skeletal muscle, bone marrow, and synovial tissues. Synovial fluid-derived mesenchymal stem cells (SF-MSCs) are recently identified as a new member of MSC families and also have the general multipotency as other common MSCs. Although SF-MSCs recently have been regarded therapeutic cells for bones, tendons, and musculoskeletal reconstruction, this is the first research to study SF-MSCs to increase vascular endothelial growth factor (VEGF) for angiogenesis regulating microRNAs (miRs). In this study, we found that miR-146a up-regulated angiogenic factor such as VEGF by targeting neurofibromatosis type 2 (NF2) which suppresses p21-activated kinase 1 (PAK1). We observed that protein and mRNA levels of VEGF were increased in SF-MSCs overexpressing of miR-146a. SF-MSCs transfected with miR-146a mimic highly increased phosphorylation of AKT which has critical role in the regulation of vascular homeostasis and angiogenesis. MiR-146a-transfected SF-MSCs also showed the increased capillary density compared to control SF-MSCs. In TargetScan website, we identified 224 common predicted genes and confirmed that NF2 is a predicted target of miR-146a by using RT-PCR and western blotting. Furthermore, cell transplantation of miR-146a-enhanced SF-MSCs improved regenerative capacity including reparative angiogenesis, interstitial fibrosis, and functionality in ischemic myocardium. Taken together, these data suggest that miR-146 is a novel miRNA to regulate angiogenesis by targeting NF2 and provide the evidence might be an important strategy for enhancing the heart functions after SF-MSC transplantation.

F-3034

AUTOGENOUS POINT OF CARE BONE MARROW CONCENTRATE (BMC) FOR THE TREATMENT OF LUMBAR DEGENERATIVE DISC DISEASE IN SELF-PAY PATIENTS

Matthew Pouliot, Kenneth Pettine
The Orthopedic Stem Cell Institute, Johnstown, CO, United States

Background:
Current
operative treatment options for moderate to severe symptomatic lumbar
degenerative disc disease include fusion versus non-fusion technology. The use
of autogenous BMC may provide another non-surgical option for this condition.
Purpose:
The
purpose of this study is to evaluate the safety and efficacy of autogenous BMC
for the non-surgical treatment of moderate to severe lumbar degenerative disc
disease.
Study
Design:
This

is a prospective non-randomized evaluation of 61 patients who underwent autogenous BMC injection into a symptomatic lumbar disc. All patients were self-paying for the procedure.

Patient Sample: Sixty-one patients had a total of 161 lumbar discs injected with autogenous BMC. The average age was 50 years and the average BMI was 26.9.

Outcome Measures: Every patient had a pre-procedure oswestry disability index (ODI), visual analog scale (VAS), physical examination, and MRI scanning. The patients were followed prospectively at six weeks, three months, and six months with repeat MRI scanning at six months.

Methods: The procedure takes 30 minutes and consists of IV sedation utilizing Versed and IV Fentanyl with the patient in the prone position. Percutaneous aspiration of the posterior iliac wing is performed to obtain 60ml of bone marrow aspirate followed by concentration utilizing the Spine Smith ART-21 system. Depending upon the number of lumbar discs to be injected, the volume of BMC was either 3ml or 6ml. Typically, 2-3ml of BMC was injected into each symptomatic lumbar nucleus with standard two-needle discography technique. Levels to be injected were based entirely on MRI scanning.

Results: The average pre-procedure ODI was 44.7% which improved to 25.2% at six months and 27.2% at 12 months (p-value < 0.0001).
The VAS averaged 61 pre-procedure which improved to 34 at six months and 38 at 12 months (p-value < 0.0001).
Thus far, only two patients have undergone a surgical procedure following the injection of autogenous BMC.
There have been no complications associated with the iliac wing aspirate or disc injection.

Conclusion: These preliminary results utilizing autogenous BMC in a prospective evaluation of 61 patients injected at 161 disc levels with minimum six-month follow-up indicate safety and very statistical efficacy. Only two patients have undergone a surgical procedure following the injection. These six-month follow-up results indicate autogenous BMC has clinical efficacy for the non-operative treatment of degenerative disc disease in the lumbar spine.
These patients will continue to be followed for a minimum of two years.

F-3035

ACTIVATION OF NRG1/ERBB4 SIGNALING PATHYWAY IN MESENCHYMAL STEM CELLS ENHANCES CELL SURVIAL AND PARACRINE POTENTIALS AGAINST MYOCARDIAL INFARCTION

Qizhou Lian, Liang xiaoting, zhang yuelin, Tse Hung-fat

medicine, The University of Hong Kong, Hong Kong, Hong Kong

ACTIVATION OF NRG1/ERBB4 AUTOCRINE SIGNALING IN MESENCHYMAL STEM CELLS ENHANCES CARDIAC PROTECTION AFTER MYOCARDIAC INFARCTION

Xiaoting Liang, M. SC;[1,2] YueLin Zhang MD;[1,2] Hung-Fat Tse, MD, PhD.[1,2] Qizhou Lian, MD, PhD;[1,2,3]

[1]Cardiology Division, Department of Medicine, University of Hong Kong, Hong Kong; [2]Research Centre of Heart, Brain, Hormone, and Healthy Aging, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong;[3]Eye Institute , Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong

Background: Although human mesenchymal stem cells (MSC) offer great promise in the treatment of myocardial ischemic diseases, the poor viability after MSC transplantation particularly drawbacks its overall restorative capacity. In this study, we proposed that the activation of NRG1/ERBB4 signaling pathway **(**Neuregulin 1 /v-erb-a erythroblastic leukemia viral oncogene homolog 4(ERBB4) in mesenchymal stem cells would enhance cell viability and trophic factor secretion in ischemic myocardium, leading to improved cardiac protection after myocardial infarction(MI).

Method and Results: Mice bone marrow-derived MSCs were characterized with positive for CD44, CD90.2, SCA-1, and negative for the hematopoietic cell lineage-specific antigens CD34, CD45, C-kit. Also, they could be readily differentiated into adipocyte, chondrocyte and osteocyte under relative induction medium. In addition, naïve MSCs were confirm negative of ERBB4 expression. Next, enforced expression of ERBB4 in MSC was performed and the secretion of NRG1 was examined. Compared to naïve MSC, the NRG1 level was significantly increased on ERBB4 overexpressed MSCs. In turn, treating with NRG1 in ERBB4 overexpressed MSC, but not in naïve MSC, showed a higher survival capacity under hypoxia challenge. The enhanced survival of MSCs was abolished by ERBB4 antibody neutralization. In mice MI model, we found the level of NRG1 is increased higher in infarcted region than in remote normal area. MSCs were injected into border of ischemic region. One month after surgical MI and MSCs injection, heart function were evaluated by echocardiography and pressure volume loop assessments in following groups: 1). MSCs overexpressing empty vector;2). MSCs overexpressing ERBB4; 3) Placebo (saline); and 4) normal control. The result showed more ERBB4-modified MSCs can survive and penetrate into ischemic region and improve cardiac performance after injury.

Conclusion: Our data showed that activation of NRG1/ERBB4 autocrine signaling in MSCs enhanced cardiac protection after myocardiac infarction in mice. Overexpression of ERBB4 in MSCs enforces NRG1 secretion and improves cell survival and migration in ischemic myocardial region.

F-3036

THE EFFECTIVNESS OF ALLOGENIC MESENHYMAL STROMAL CELLS OF BONE MARROW IN PATIENTS WITH REFRACTORY CROHN'S DISEASE

Oleg Knyazev[1], Irina Ruchkina[1], Igor Hatykov[1], Anatoliy Konoplyannikov[2]

[1]Central Research Institute of Gastroenterology, Moscow, Russian Federation, [2]Medical Radiological Research Center, Obninsk, Russian Federation

Crohn's disease (CD) is a chronic inflammatory disease of the gastrointestinal tract with recurrent nature of the flow. The frequency of exacerbations is approximately 20-25% at 1 year and 75% for 3 years. If the remission lasted less than 12 months, there is a 65% chance that the aggravation comes in the next 18 months. For the duration of remission for 12 months or more the likelihood of an explosion in the next 18 months is reduced to 20%.

Aim. To evaluate the influence of culture of allogeneic mesenchymal stromal cells (MSCs) of bone marrow for the duration of remission in patients with refractory CD.

Materials and Methods. The first group of patients with CD (n=30)

received MSCs, the dose of prednisone was not more than 20 mg/day. The second group of patients (n=30) received standard anti-inflammatory drug therapy of 5-aminosalicylic acid (5-ASA) and glucocorticosteroids (GCS). Age of patients ranged from 19 to 49 years (Me-36 years). The disease was of moderate and high activity, length of damage - ileokolit, ileitis and colitis, the observation time ranged from 42 to 68 months. Clinical activity was assessed by the Crohn's disease activity index (CDAI). The culture of allogeneic MSCs injected drip at 2.5 million per 1 kg of body weight (0-1-26 weeks).
Results. CDAI in the 1-st group was 242,6±11,7 points, in the 2-nd 240,9±12,9 points (p=0,83), CRP levels in 1-st group was 29,3±6.4 mg/l, the 2-nd - 27,8±4,8 (p=0,47). After 1 year of follow-CDAI in 1-st group was 70,0±11,0 points, in the 2-nd - 133,8±22,2 points (p<0,001), CRP levels in 1-st group was 6,36±1,5 mg/l, in the 2-nd - 12,2±2,9 (p<0,001). After 2 years CDAI 1-st group was 99,6±19,3 points, in the 2-nd - 147,1±22,1 points (p<0,001), CRP levels in 1-st group was 16.0±6,0 mg/l, in the 2-nd - 18,8±4,4 (p=0,156). After 3 years, the CDAI in 1-st group was 110,5±21,9 points, in the 2-nd - 180,6±20,3 points (p<0,001), CRP levels in 1-st group was 10.9±2,6 mg/l, in the 2-nd - 16,9±3,0 (p<0,001). After 4 years - the CDAI in 1-st group was 120,0±22,3 points, in the 2-nd - 208,7±17,6 points (p<0,001), CRP levels in 1-st group was 11,3±2,6 mg/l, in the 2-nd - 15,5±2,4 (p<0,001). After 5 years - the CDAI in 1-st group was 126,0±23,8 points, in the 2-nd - 248,7±14,6 points (p<0,001), CRP levels in 1-st group was 12, 3±2,8 mg/l, in the 2-nd - 19,5±3,1 (p <0,001). In the first group of patients in remission after 1, 2, 3, 4, and 5 years was kept at 70%, 56.6%, 50%, 46.7% and 43.3%, respectively. In the second group of patients at 1, 2, 3, 4, and 5-year remission was maintained at 36.6%, 26.6%, 13.3%, 6.67% and 6.67%, respectively. Complete healing of the intestinal mucosa in 60% of patients in the first group during the 1st year of observation, after 5 years - 26.7%. Over the entire period of observation never there were no malignant transformation, life-threatening infectious complications and death.
Conclusions. Transplantation of MSCs contributes to longer-term clinical and endoscopic remission in patients with refractory Crohn's disease compared with therapy with corticosteroids.

F-3037

MESENCHYMAL STEM CELLS PROMOTE NEUROPROTECTION AND AXONAL REGENERATION IN A MODEL OF OPTIC NERVE CRUSH

Louise A. Mesentier-Louro, Camila Zaverucha-do-Valle, Ana Beatriz Padilha de Figueirêdo, **Gabriel Nascimento-dos-Santos**, Almir J. Silva Junior, Ana L. Torres, Bruno D. Paredes, Camila Teixeira, Fernanda Tovar-Moll, Rosália Mendez-Otero, Marcelo F. Santiago
Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Bone marrow derived cells have been used in different animal models of neurologic diseases. In this study we have investigated the therapeutic potential of mesenchymal stem cells (MSC) injected into the vitreous body in a model of optic nerve injury. Adult (3-5 months old) Lister rats underwent unilateral optic nerve crush followed by MSC or vehicle injection into the vitreous body. Sixteen and 28 days after injury, RGC survival was evaluated assessing the number of Tuj1 or Brn3a-positive cells in flat-mounted retinas, and optic nerve regeneration was investigated after anterograde labeling of the optic axons with cholera toxin B conjugated to Alexa 488. MSC were labeled before injected with a fluorescent dye or with superparamagnetic iron oxide nanoparticles (SPIONS) which allowed us to track the cells in vivo by magnetic resonance imaging. Transplanted MSC remain in the vitreous body and are found

in the eye for several weeks. Cell therapy with MSC increase the number of Tuj1 and Brn3a positive cells in the retina and the number of axons distal to the crush site both at 16 and 28 days after optic nerve crush. In summary, MSC protects RGC and stimulates axon regeneration after optic nerve crush. The long permanence of the transplanted cells in the eye may account for the sustained effect observed. Further studies are necessary to elucidate mechanisms of action of MSC in the visual system.

Mesenchymal Cell Lineage Analysis

F-3043

MOUSE TELOMERASE REVERSE TRANSCRIPTASE (MTERT) EXPRESSION MARKS QUIESCENT ADIPOGENIC PROGENITOR CELLS IN ADIPOSE TISSUE STROMAL VASCULAR FRACTION

Matthew D. Lynes[1], Diana L. Carlone[2], David T. Breault[2], Yu-Hua Tseng[1]

[1]Integrative Physiology and Metabolism, Joslin Diabetes Center, Boston, MA, United States, [2]Boston Children's Hospital, Boston, MA, United States

Most mammalian stem cell populations are thought to be maintained in a quiescent state, responding to extrinsic cues by proliferating to replace damaged tissue. Coexistence of quiescent and proliferative stem cells has been demonstrated in several tissue types including adipose tissue. Normal adipose tissue turnover is relatively fast with between 1-5% of adipocytes replaced daily by progenitor cell differentiation and proliferation, however a small population of label retaining cells remains after a 2-month chase. This suggests the presence of a quiescent population of cells that does not contribute to normal tissue maintenance; however the identity of these rare cells remains unclear. Loss of telomeric DNA beyond a critical threshold induces senescence in most somatic cells. The maintenance or induction of telomerase expression and activity in quiescent cells provides a means of preventing cellular senescence that may be relevant for tissue self-renewal and could be used to identify these tissue resident stem/progenitor cells. High fat diet is associated with decreased mTert expression in adipose tissue. Furthermore, brown adipose tissue (BAT) mTert expression is negatively regulated by cold induced activation, while mice maintained at thermoneutrality had higher mTert expression in BAT. Using mTert-GFP reporter mice, we detected mTert expression in a small (approximately 0.5-1%) subset of white and brown adipose stromal vascular cells by fluorescence activated cell sorting and histological analyses. Lineage tracing studies using mTert-CreER mice revealed a small population of mTert positive cells in brown and white adipose tissue immediately after the pulse. After a 6-month chase, we detected less than 1% of adipose cells arise from mTert positive lineage during normal tissue turnover. Using in vitro lineage tracing experiments, we demonstrated that mTert positive stromal vascular cells are able to differentiate into lipid-laden cells that stain positive for the lipid droplet-associate protein perilipin, suggesting that they are adipogenic progenitors. These data demonstrate the expression of mTert in adipose tissue, and suggest the presence of a quiescent mTert+ population of adipogenic progenitor cells residing in brown and white fat.

F-3044

MORPHOLOGICAL DEFINING LN VIVO LOCALIZATION OF HUMAN ADIPOSE DERIVED MESENCHYMAL STEM CELL

JaeJin Cho[1], Young-joon Ryu[3], Taejun Cho[1], Jin-Young Choi[4], Dong-Sup Lee[2]

[A]School of Dentistry, [B]School of Medicine, [1]Seoul National University, Seoul, Korea, Republic of, [2]Department of Pathology, Asan Medical Center, Seoul, Korea, Republic of, [3]Department of Oral and Maxillofacial Surgery, Seoul National University Dental Hospital, Seoul, Korea, Republic of

Human adipose derived mesenchymal stem cells (hADSCs) can be a source an autologous cell therapy, due to their indispensable regenerative ability. However, their detail cytological or phenotypic information are still unclear. Therefore, we investigated to file up and compared cellular size, morphology, ultrastructure, immunohistochemical (IHC) expression profile both of isolated hADSCs and the cells located in the tissue. Furthermore, We traced

and identified in vivo localization of the cells. On light microscopic examination, hADSCs became spindle shaped morphology after 4 passages in low power view. However, there are various size, nuclear contours, and cytoplasmic textures on high power view. To define morphology, transmission electron microscopy (TEM) examination was done. hADSCs commonly showed ultrastructural characteristics of primitive mesenchymal cells, including relatively high N/C ratio, prominent nucleoli, immature cytoplasmic organelle, numerous pseudopods. Few endothelial cells, smooth muscle cells, or pericytes are noted. Some cells show various amount of lamella body or vacuole with or without small lipid droplet. On IHC staining, PDGFR and CD10 continuously expressed in most hADSCs regardless individual or passage, but expressions of SMA, CD68, and Oct4 or c-kit were variable. On IHC staining of adipose tissue, same immunophenotypic expressed cells with hADSCs were found mainly at perivascular adventitia of small to medium sized vessels. On TEM examination, cells having similar ultrastructural morphology were found intensively at perivascular adventitia of small to medium sized vessels. In summary, hADSCs are heterogenous cell population, showing primitive mesenchymal cells. It is isolated mainly from at perivascular adventitia of medium to small sized vessel. So called “hADSCs” composed of endothelial cell, tissue macrophages, and smooth muscle cells progenitor, and real mesenchymal progenitor cells could intermingle in cell therapy products in used isolation method. These results give basic essential phenotypic information about hADSCs as cell therapy products, which are helpful information to evaluate preclinical or clinical cell therapy. To isolate defined cell therapy product, further study for more specific isolation method is necessary

F-3045

THE CHARACTERIZATION OF HUMAN ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS FROM DIABETIC DONORS

Thuy N. Trinh[1], Kenichi Kimura[1], Fujio Sato[2], Toshiharu Yamashita[1], Georgina Salazar[1], Shonosuke Matsushita[2], Yuzuru Sakakibara[2], Osamu Ohneda[1]

[A]Regenerative medicine and stem cell biology, [B]Cardiovascular Surgery, [1]University of Tsukuba, Tsukuba, Japan

Mesenchymal stem cells (MSCs) are promising resources for regenerative medicine. Adipose tissue-derived MSCs (AT-MSCs) are easily isolated and used for auto-transplantation. This study focuses on identifying the characteristic differences between AT-MSCs from diabetic donors (dAT-MSCs) and non-diabetic donors (nAT-MSCs).
Diabetes mellitus is a chronic and complex disorder of common relevance to environmental and life style factors. There were more than 250 million people had diabetes mellitus and the number of patients is estimated increase almost double by 2030. The major problem caused by the cells in body lost their abilities of response to insulin signal, resulting in poor glucose control and degenerative complications. Diabetic complications are cased by long term uncontrolled hyperglycemia and most of them are thought to be the result of problems with blood vessels. However, there were a few reports about the influence of diabetes mellitus to MSCs.
We found that dAT-MSCs had similar capacities to differentiate into adipocytes, osteoblasts, and chondrocytes as nAT-MSCs. The expressions of Runx2 and ALP, the early osteogenic markers, were not significantly different between dAT-MSCs and nAT-MSCs. However, the expressions of PPARg2 and adiponectin, the early adipogenic markers, were significantly higher than those of nAT-MSCs. Cell growth activity of dAT-MSCs was also similar as that of nAT-MSCs. On the other hand, dAT-MSCs showed more impairment of wound healing in mouse flap model, compared with nAT-MSCs. Immunohistochemical analysis revealed that CD45 positive cells were increased in the flap sites of dAT-MSCs transplantation. Additionally, Lectin-binding TRIC analyses showed that the abnormal vessel formation in the flap sites with dAT-MSCs transplantation. Furthermore, the expression of angiogenic factors such as bFGF, VEGF, and Ang-1 were significantly up-regulated in dAT-MSCs compared to those of nAT-MSCs under hypoxic conditions. These results suggest that dAT-MSCs may work abnormally under hypoxic stress leading to vascular permeability and abnormal microvascular formation, and chronic inflammation in tissue repair, but not impairment in differentiation and proliferation. This work provides in vitro and in vivo evidence that there were quite different characteristics between dAT-MSCs and nAT-MSCs.

F-3046

ANALYSIS OF ACTIN CYTOSKELETON IN STEM CELLS OF AUTISTIC PATIENTS: A PRELIMINARY STUDY

Karina Griesi-Oliveira, Danielle Y. Sunaga, Maria Rita Passos-Bueno
Genetics and Evolutionary Biology, Bioscience Institute - University of Sao Paulo, Sao Paulo, Brazil

Autism spectrum disorders (ASD) are characterized by deficits in reciprocal social interaction and communication as well as by the presence of repetitive behaviors. ASD are genetically heterogeneous, which has been made difficult the development of molecular tests for diagnosis and the understanding of their etiology. On the other hand, it is interesting to note that several genes implicated in the etiology of ASD are part of common molecular pathways or mechanisms, indicating that these different genetic alterations may cause simmilar effects during neurogenesis, leading to the same behavioral features. One of such common mechanisms may be the cytoskeleton dinamics regulation, which is essential for organization and plasticity of dendritic spines as well as axonal growth and guidance. However, the relationship between these mechanisms and the etiology of ASD has been poorly explored in the literature, specially in their functional aspects. Previous results from our group have suggested a disturbance in cytoskeleton dynamics in cells from autistic patients, which showed a deregulated expression of RhoGTPases. Thus, here we aimed to investigate the actin cytoskeleton dinamics regulation in stem cells from human exfoliated deciduous teeth (SHED) from ASD idiopathic patients. First, we incubate the cells of 8 patients and 3 controls for 24h using Rho kinase inhibitor (ROCKi) in order to depolymerize the microfilaments. To induce actin cytoskeleton reconstruction, ROCKi was washed out and the cells were treated with EGF (epidermal growth factor), a drug that activates Cdc42, one of the main small RhoGTPases that regulate actin polymerization. The percentage of cells presenting actin filaments were counted at 20, 40, 60 and 80 minutes after EGF application. Our preliminary results showed that half of the patients presented a significantly greater percentage of cells with recovered actin filaments at 20, 40 and 60 minutes compared to controls (t=20min - patients: 30.7±0.02%, controls: 18.67±0.03%; t=40min - patients: 53.75±0.05%, controls:32.67±0.03%; t=60min - patients 78±0.05%, controls: 51±0.003%; unpaired t-test $p<0.05$ for all time points), although at 80 minutes no significant difference was observed. This results suggest that, at least for a group of ASD patients, the dynamics of actin polymerization induced by cdc42 activation is faster compared to control individuals. Induction of actin polymerization by other drugs should be tested in order to verify if the other patients may have cytoskeleton dynamics deregulated when this is triggered by RhoGTPases such as RhoA and Rac. We believe that investigation of such aspects in neuronal cells derived from induced pluripotent stem cells is worthwhile. Finally, we consider that this study can contribute for the understanding of the common molecular mechanisms involved in ASD etiology as well as for the finding of new drugs that can help in the treatment of these patients.

F-3047

AUTOTAXIN STIMULATES HUBC-MSC MIGRATION THROUGH LPA RECEPTOR 1, 3-DEPENDENT GαQ-MEDIATED PKC/GSK3β/β-CATENINE PATHWAYS

Jung Min Ryu, Young Bin Baek, Myung Sun Shin, Ji Hoon Park, Ho Jae Han
Seoul National Univ, Seoul, Korea, Republic of

Autotaxin (ATX) is a key enzyme that regulates lysophosphatidic acid (LPA) levels in biological fluids, which exerts a wide range of cellular functions including embryonic development and cell migration. However, the biological role of ATX in human umbilical cord blood-derived mesenchymal stem cell (hUCB-MSC) migration and signaling mechanisms remain to be fully elucidated. In the present study, we observed that ATX and LPA treatment increased MSC migration in a time- and dose-dependent manner. Indeed, LPA receptor 1-5 were expressed in MSC and LPA receptor 1-3 located in lipid raft. In experiments to investigate whether the ATX-induced migration is depended on LPA and its receptors, ATX stimulates the MSC migration through LPA production and LPA receptor 1, 3 dependent manners. Furthermore, LPA treatment decreased binding with LPA receptor 1-3 and Gα subunit i, q, and 12. LPA treatment increased the Ca2+ influx and PKC phosphorylation, which were blocked by Gαq siRNA transfection, suggesting that the Gαq has an important role in LPA-induced PKC activation. LPA also increased the GSK3β phosphorylation and β-catenine activation in a time-dependent manner. In addition, LPA induced translocation of β-catenine from cytosol to nuclear, which was inhibited by PKC inhibitors (staurosporine and bisindolylmaleimide I). LPA stimulates the binding of β-catenine on E-box located in promoter of CHD1 gene (E-cadherin). Moreover, ATX and LPA-induced decrease in MSC migration through downregulation of E-cadherin expression and upregulation of MMP-2, -9 expressions were blocked by β-catenine specific siRNA transfection. In conclusion, ATX stimulates the hUBC-MSC migration through LPA receptor 1, 3 dependent decrease of E-cadherin expression via PKC/GSK3β/β-catenine pathways.

F-3048

DISTINCT BILINEAGE DIFFERENTIATION ABILITY AND PLASTICITY CORRELATE WITH MOLECULAR PHENOTYPE OF TWO HUMAN CLONAL BONE MARROW STROMAL CELL LINES

Mona A. Elsafadi[1], Manikandan Muthurangan[2], Abdullah Aldahmash[2], Amer Mahmood[1]

[1]Department of Endocrinology, University of Southern Denmark, Odense, Denmark, [2]Department of Anatomy, King Saud Universtiy, Riyadh, Saudi Arabia

The ability of bone marrow stromal cells (BMSCs) to differentiate into osteoblasts is being exploited in cell-based therapy for repair of bone defects. However, the molecular phenotype of ex vivo BMSCs predicting their bone-forming and plasticity capacity is not yet well elucidated. Thus the aim of this study was to define an ex vivo molecular phenotype of two hBMSCs populations that exhibited variant commitment to osteoblastic and adipogenic lineages and have distinct plasticity potential.

Here we subcloned two BMSCs populations: one has higher bilineage differentiation potential (HD-hBMSC-TERT, termed “HD”) and the other has less (LD-hBMSC-TERT, termed “LD”) from hBMSC-TERT (telomerase-immortalized hMSC line). While HD and LD have different morphological appearance, they both showed similar expression level of MSC specific genes, known MCS-surface markers, and immunocytochemical patterns. Upon differentiation, HD cells were more osteoblast-committed cells as proven by matrix mineralization , increased ALP activity, and high gene expression of osteoblastic markers (ALP, Osteopontin, Runx2, Twist, and BMP4). In addition, extensive accumulation of lipid droplets upon adipocytic differentiation in HD was associated with gradual up-regulation in the gene expression of adipocytic markers including PPARγ, aP2, LPL, and Adiponectin.

By employing DNA microarray, we found many mesodermal and mesenchymal specific genes up-regulated in both HD and LD. However skeletal muscle, mineralization and bone-related genes were more up-regulated in HD cells including ALPL, POSTN, SSP1, TNFRSF11B, IGFBP5, NOV, and CRYAB, proving the pronouncement of osteoblastic phenotype of these cells. In contrast, diminished proliferation and differentiation rate of LD was associated with significant up-regulation of SFRP1 and SFRP2 (>10 fold) that are involved in Wnt pathway inhibition, suppression of bone formation, and in tumor and proliferation suppression.

Interestingly, these cells exhibit high plasticity potential proved by their differentiation into osteoblasts after commitment into adipogenic lineage. Upon transdifferentiation, matrix mineralization and elevation of relative osteogenic gene expression levels (ALP, RUNX2 and OPN) were associated with gradual down regulation to extremely low level of adipogenic markers (PPARγ and LPL). By performing microarray data analysis, we found 763 common genes were down regulated (71%) upon osteoblast differentiation following their up-regulation (55%) upon adipo-

cyte differentiation. GO analysis showed that most of the extremely down regulated genes in transdifferentiated cells were involved in lipid metabolism and adipogenesis.
In conclusion, our data shows that our clones of hBMSC have different differentiation ability and plasticity that correlate with molecular phenotype. Hence, molecular signature of hBMSC is a suggested tool for prospective identification of osteogenic BMSC populations. Understanding regulating pathways and molecular signature that mediate the transdifferentiation between osteoblasts and adipocytes is relevant to the development of therapeutic control of bone loss in osteoporosis. Identifying markers that control cross-lineage commitment among different cell types would be useful not only in shedding light on fundamental mechanisms regulating development, and pathogenesis of osteogenic disorders but also in the quality control of osteoblastic cells before use in therapy.

F-3051

EFFECTS OF SERIAL PASSAGE ON THE CHARACTERISTICS AND CHONDROGENIC DIFFERENTIATION OF CANINE UMBILICAL CORD MATRIX DERIVED MESENCHYMAL STEM CELLS

Chan-Lan Kim, Keum Sil Lee, Sang-Ho Cha, Hye Won Kang, Jae Young Song, Kyung Woo Lee
Department of Animal and Plant Health Research, Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang-si, Gyeonggi-do, Korea, Republic of

Effects of Serial Passage on the Characteristics and Chondrogenic Differentiation of Canine Umbilical Cord Matrix Derived Mesenchymal Stem Cells

Chan-Lan Kim, Keum Sil Lee, Sang-Ho Cha, Hye Won Kang, Jae Young Song, Kyung Woo Lee
Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang 6-dong, Anyang-si, Gyeonggi-do 430-757, Korea

ABSTRACT: Mesenchymal stem cells (MSCs) are often known to have a therapeutic potential in the cell-mediated repair for fatal or incurable diseases. In this study, canine umbilical cord MSCs (cUC-MSCs) were isolated from umbilical cord matrix (n = 3) and subjected to proliferative culture for 5 consecutive passages. The cells at each passage were characterized for multipotent MSC properties such as proliferation kinetics, expression patterns of MSC surface markers and self-renewal associated markers, and chondrogenic differentiation. In results, the proliferation of the cells as determined by the cumulative population doubling level was observed at its peak on passage 3 and stopped after passage 5, whereas cell doubling time dramatically increased after passage 4. Expression of MSC surface markers (CD44, CD54, CD61, CD80, CD90 and Flk-1), molecule (HMGA2) and pluripotent markers (sox2, nanog) associated with self-renewal was negatively correlated with the number of passages. However, MSC surface marker (CD105) and pluripotent marker (Oct3/4) were decreased with increasing the number of subpassage. cUC-MSCs at passage1 to 5 underwent chondrogenesis under specific culture conditions, but percentage of chondrogenic differentiation decreased with increasing the number of subpassage. Collectively, the present study suggested that sequential subpassage could affect multipotent properties of cUC-MSCs and it needs to be addressed before clinical applications.
Keywords: **Mesenchymal stem cell, Umbilical cord matrix, Canine, Multipotent, Differentiation**

F-3052

CELL SURFACE MARKER PROFILING OF ADIPOSE-DERIVED STEM CELLS FROM HUMAN SUBCUTANEOUS AND VISCERAL FAT DEPOTS

Wee Kiat Ong[1], Edmund Chan[2], Sue-Anne Toh[2], Weiping Han[3], **Shigeki Sugii**[3]
[1]Singapore Bioimaging Consortium, Singapore, Singapore, [2]National University of Singapore / National University Health System, Singapore, Singapore, [3]Singapore Bioimaging Consortium / Duke-NUS Graduate Medical School, Singapore, Singapore

White adipose tissues of subcutaneous and visceral depots differ in their pathophysiological contribution to metabolic homeostasis. The subcutaneous fat depot physiologically stores excess lipids thus preventing their deposition into other organs. Visceral fat accumulation, on the other hand, leads to pathological metabolic profile due to dys-

function in lipid storage. Increasing evidence suggests that this can be attributed to difference in inherent properties of the adipose-derived stem cells (ASCs) from the two fat depots. Currently, little is known about the difference in identity of ASCs from the two fat depots. We isolated and cultured subcutaneous and visceral (omental region) ASCs from human subjects. As expected, ASCs from subcutaneous fat differentiate better into mature adipocytes than those from visceral fat by the standard in vitro adipogenesis protocol. High content screening assay of over 200 human cell surface markers was performed to identify potential depot-specific cell surface markers of ASCs. Several candidates that showed differential immunofluorescence signals in terms of signal intensity and cell percentage were selected for further study. Identification of such markers would allow us to differentially isolate, visualize and characterize ASCs in the depot-specific manner.

F-3053

STEM CELLS FROM CANINE DECIDUOUS TOOTH - SPONTANEOUS POTENTIAL IN OSTEOBLAST LINES

Dayane Alcântara, Márcio Nogueira Rodrigues, Paula Fratini, Rafael Cardoso Carvalho, Sonia Elisabete Alves de Lima Will, Maria Angelica Miglino
Department of Surgery, School of Veterinary Medicine. University of São Paulo, São Paulo, Brazil

Cell therapy has been studied in veterinary medicine and dogs are commonly used as animal models for regenerative research. Mesenchymal stem cells (MSCs) have a capacity of self-renewal and multilineage differentiation. Search about MSCs lead to study of several cell types in every organs and tissues. Dental pulp has a promising population of stem cells with tissue regeneration potential. The aims of this study were to establish, characterized the canine deciduous tooth stem cells (cDTSC), and compare their potential between the initial (P1) and 10^{th} passages culture. Premolar of six-month-old dog was used to obtained cDTSC. The
tooth was washed in saline buffer with 5% of antibiotics (penicillin and
streptomycin) for three times and pulp was extracted of the chamber. It was performed explants methods and the fragments were plated with fetal bovine serum (FBS) for 4h. These cells were cultured in α minimum essential medium supplemented with 10% FBS, 1% of antibiotics (penicillin and streptomycin), 1% of non-essential amino acids and L-glutamine. The morphology of these cells was photodocument. It was analyzed the homing cellular in embrionated chicken eggs, markers expression by flow citometry and imunocitochemistry. The cells in 10^{th} passage (P10) was stained with Von Kossa, Sudan II Scarlate and Masson. cDPSC has fibroblast like and the analyses of homing cellular showed migration of these cells from the beak of the chicken fetus. Along the culture, cDPSC were differentiated into osteoblasts spontaneously, comproved by Von Kossa stain. These cells expressed Oct4, Nanog, Stro-1, CD90, CD105 and VEGF but the expression was decreased along the culture. They were positive for CD34 and CD45 in the initial culture but were negative in P10. We concluded that these cells are easy to isolate and have a stem cells potential; The expression of markers decrease along the culture, and the cDTSC differentiate spontaneously in bone lines.

F-3054

DIFFERENT PATTERN OF SOX2 EXPRESSION AS EMBRYONIC MARKER IN EQUINE ADIPOSE AND MARROW-DERIVED MESENCHYMAL STEM CELLS

Abbas Abavisani, Adham Fani Maleki, Morteza Zahedi, Faezeh Alipour
Basic Sciences, Veterinary Faculty & Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran, Islamic Republic of

Bone marrow (BM) and adipose tissue (AT) are two main sources of mesenchymal stem cells (MSCs) for basic and clinical studies. MSC populations obtained from different tissues exhibit significant differences in gene expression profile which should be taken into consideration when planning their use in clinical protocols. In spite of more than a decade of investigation, little is known about the exact molecular mechanisms regulating multipotency or pluripotency state of MSCs. Our aim was to compare expression status of SOX-2 in BM-MSCs and AT-MSCs, as a factor that involve in maintenance of the pluripotent state of embryonic stem cells. For this purpose, MSCs were isolated

from adipose tissue and bone marrow of 3 mares, cultured and passaged until passage3 (P3). Differentiation potential of P3-MSCs was confirmed by their osteogenic, chondrogenic and adipogenic differetiation in vitro. Total RNA was extracted from P3 cells and reverse transcriptase- polymerase chain reaction (RT-PCR) was done. We found that SOX-2 is expressed in cultured equine BM- MSCs, but not in AT- MSCs. This indicates that although BM- MSCs and AT-MSCs seem to be closely related, there are differences in their biological characteristics which can affect their in vivo biological behavior. More investigation is needed to clarify if sox-2 expression is obligatory for BM-MSC pluripotency.

F-3055

MOLECULAR SIGNATURE OF HETEROTOPIC OSSIFICATION BY DYSREGULATED MICRORNAS IN ORTHOPAEDIC COMBAT BLAST INJURY

Youngmi Ji[1], Emily Shin[2], Matthew Kluk[2], Jared Vogler[2], Carl Cirino[1], Nayha Patel[1], Wesley Jackson[3], Leon Nesti[1]
[1]NIH, Bethesda, MD, United States, [2]Walter Reed National Military Medical center, Bethesda, MD, United States, [3]USUHS, Bethesda, MD, United States

Heterotopic Ossification (HO), characterized by ectopic bone formation in soft tissues, is a complication that can accompany musculoskeletal injury, and now frequently occurring within the military population due to extensive orthopaedic combat trauma. Although the mechanism of traumatic HO is unclear, evidence suggest that progenitor cells within the muscle become dysregulated by conflicting wound-healing responses and initiate osteogenesis. In this context, we have identified a population of mesenchymal progenitor cells (MPCs) in traumatized muscle tissue that are capable of osteogenic differentiation. To understand the molecular basis of osteogenic differentiation, we explored with the hypothesis that the presence of miRNAs following traumatic muscle injuries could be sufficient dyregulate expression of targets genes and thus direct naive progenitor cells away from the process of normal muscle regeneration and instead allow pathological differentiation to occur. Our specific aims were (1) to generate list of differentially expressed miRNAs from comparisons between patient groups with/without HO, (2) to evaluate the function of candidate miRNAs in MPCs during osteogenesis in vitro, (3) to generate a molecular signature of miRNAs in MPCs using RNA-Seq, and (4) to generate a molecular signature of HO by comparing data of MPCs with manipulated levels of miRNA and patient samples with/without HO. Our preliminary data suggest that overall differentially expressed miRNAs with roles in cell differentiation and development were up-regulated in HO positive tissues using real-time miRNA PCR array analysis. A sub-set of these (miR-1, miRNA-206, miR-133a/b, miR-26a, miR-125b) were further validated. MPCs manipulated with these miRNA expression showed more osteogenesis compare to control cells. Our data suggest that dyregulated miRNAs may play a role in abnormal wound healing process that results in HO following traumatic injury. Finally, the molecular signature of the target genes of these miRNAs can potentially be developed for therapy to prevent HO from extensive orthopaedic combat blast injury.

Mesenchymal Stem Cell Differentiation

F-3057

BDNF INDUCED GABAERGIC NEURONAL DIFFERENTIATION IN HUMAN MESENCHYMAL STEM CELLS

jihea yu, junghwa seo, jiyeon kim, yanghyun cho, sungrae cho
Department & Research Institute of Rehabilitation Medicine, Yonsei University College of Medicine, seoul, Korea, Republic of

Objective: Mesenchymal stem cells (MSCs) have been derived from many different sources including bone marrow (BM), umbilical cord blood (UCB) and adipose tissue (AT). We tested the hypothesis that human MSCs from different tissues may differentiate into GABAergic neurons in response to brain-derived neurotrophic factor (BDNF). This potential for GABAergic neuronal differentiation was investigated in MSCs from BM, UCB, and AT.

Methods: The UCB-, BM-, and AT-MSCs were plated onto poly-D-lysine/laminin with BDNF, and were compared with control MSC cultures plated without BDNF. The GABAergic neuronal differentiation was detected by morphological criteria. For comparison of GABAergic differentiation potential, RT-PCR was carried out with nestin and GAD67, a GABAergic neuronal marker, and immunocytochemistry was detected as the expression of βIII-tubulin and GABA.
Results: After 7 days of GABAergic induction, the three types of MSCs assumed thinner and longer shapes, similar to neuronal cells, in the presence of BDNF, while control cultures plated without BDNF retained the flat morphology. The BDNF-treated groups of BM-, UCB-, and AT-MSCs expressed GAD67 at higher levels than control groups, while nestin expression did not change in any BDNF group. We detected very low levels of GAD67 mRNA expression in AT-MSCs that underwent GABAergic neuronal differentiation. However, BM-MSCs that differentiated showed significant increases in GAD67 expression. The percentage of GABA+ cells / DAPI+ cells in BDNF groups was increased compared with the controls of BM-MSCs (9.88 ± 2.18 % versus 21.1 ± 2.66 %), AT-MSCs (5.77 ± 1.46 % versus 9.73 ± 1.65%), and UCB-MSCs (5.92 ± 2.08 % versus 7.69 ±1.95 %).
Conclusion: We conclude that BM-MSCs are currently the most ideal cell source for human MSCs into GABAergic differentiation.

F-3058

COMPARATIVE CARDIOGENIC AND ANGIOGENIC POTENTIAL OF MESENCHYMAL STEM CELLS ISOLATED FROM DIFFERENT POST-BIRTH TISSUES.

Paz González[1], Lorena Salazar[2], Rafael Armijo[3], Paulina Maffud[2], Sylvia Flores[2], Diego Seguel[4], Fernando Figueroa[1], Jorge Bartolucci[2], Maroun Khoury[2]

[1]Faculty of medicine, Laboratory of miRNA and stem cells, Universidad de Los Andes, Santiago, Chile, [2]Universidad De Los Andes, Santiago, Chile, [3]Universidad Santo Tomas, Santiago, Chile, [4]Universidad de Santiago de Chile, Santiago, Chile

Mesenchymal stem cells (MSCs) have been applied in a range of animal models of disease and human conditions including acute myocardial infarction and heart failure. A critical limitation in the field, is the availability of cell sources that are both accessible and effective for cell therapy. For this, post-birth tissues are considered as an ideal source for MSC because of their accessibility and lack of ethical conflicts. However, MSCs can be isolated from different placental tissues and areas such as the amniotic membrane (AM), chorion (Ch) and Decidua (Dc).This study focuses on assessing the distinct cardiogenic and angiogenic differentiation capability of human MSCs derived from umbilical cord (UC) and the different placental tissues.
Methods: The myocardiac differentiation was induced by 5-azacytadine (5-AZA), or by co-culture with rat cardiomyocyte, and the expression of the differentiation factors analyzed by quantitiative PCR . The angiogenesis potential was determined using a matrigel assay, where sample were directly analyzed for the formation of tubes, or by the indirect effect of their conditioned supernatant on angiogenesis of endothelial cells. The secretion of growth factors was quantified by ELISA.
Results: While basal expression levels of transcription factors associated with cardiac lineage such as GATA-4 and Nkx2.5 where only detected in Ch-MSCs, the expression of cardiac myosin was detected after AZA treatment in all tested sources. Ch-MSCs showed 2 and 1000 x higher expression of cardiac myosin when compared to the Dc-MSCs and UC respectively. Furthermore, the in vitro angiogenic assay showed a markedly higher potential for tubules formation of MSCs from chorion and decidua, while the reorganization into tubules from UC-MSCs was largely reduced. Moreover, the Ch-MSCs conditioned medium showed the highest angiogenic enhancing effect on cultured endothelial cells from the umbilical cord (HUVEC). Consistent with these findings, Ch-MSCs were the only source to express all three measured growth factors (VEGF, FGF-1 and HGF). FGF-1 and HGF were detected at lower levels in UC-MSCs conditioned media, while Dc-MSCs were poor secretors and only moderate level of VEGF was detected.
Conclusion: These results suggest that among different post-birth tissues, chorion derived MSCs are the most potent candidate for cardiovascular regenerative cell therapy approaches. The in vivo potential of these cells is under evaluation in a related animal model.

F-3061

CARDIOMYOGENIC DIFFERENTIATION IN HUMAN ADIPOSE DERIVED MULTIPOTENT STROMAL CELLS (ASCs)

Greta Minonzio[1], Mattia Corazza[1], Luca Mariotta[1], Mauro Gola[2], Gianni Soldati[1]
[1]Swiss Stem Cell Foundation, Lugano, Switzerland, [2]Stem Cell Research, Lugano, Switzerland

The goal of this project is to differentiate adult human Adipose tissue-derived multipotent Stem Cells (ASCs) into cardiomyocytes or close progenitors. For that two innovative approaches have been investigated: the use of human growth factors in a defined serum-free culture medium and the application of mechanical and electrical stimuli to mimic the physiological environment of the heart. Both studies aim to develop stem cell-based therapies for the cure of cardiovascular diseases. This appears to be a challenge due to the peculiar functional and anatomical heart properties. Cardiomyocyte regeneration is limited in adult life and therefore the identification of a putative source of cardiomyocyte progenitors is of great interest to provide usable in vitro models and new perspectives for regenerative therapies.
Here we present a new isolation protocol to obtain ASCs according with the standard GMP-procedure which allows to obtain a substantial number of cells for the clinical application. The starting material was obtained from subcutaneous adipose tissue collected during an elective liposuction procedure. The isolated ASCs were characterized by flow cytometry and expanded until second passage. Different cocktails of growth factors, cytokines and culture conditions were tested for their ability to induce cardiomyogenesis. The goal was to identify the right combination of those factors and their timing of application in order to obtain cardiomyocytes from ASCs. After testing the effect of those cardiogenic cocktails, the one which gave the best result in respect to induction of 12 cardiac markers (e.g. Nkx2.5 and Mef2C) has been selected. Surprisingly non induced ASCs showed to express half of the selected genes demonstrating and explaining the high potential and the inherently capacity to differentiate into cardiac precursors of ASCs. Furthermore we analysed the morphological changes of ASCs after 3 weeks of differentiation with the use of electron microscopy and immunofluorescence staining. With the selected cocktail the differentiation was highly reproducible, as shown by the results of 15 patients.
In conclusion our results show that we found a new cardiomyopoietic cocktail medium.
We will test a new approach based on the application of an electrical field aimed to stimulate the maturation of cardiomyocytes. For that a customized device has been built to allow the culture of engineered 3D cardiac tissue.

F-3062

RESPONSE OF ADIPOSE DERIVED STEM CELLS IN CHONDROITIN SULFATE RESERVOIR EMBRACED ELECTROSPUN MEMBRANES FOR IN VITRO ENGINEERING OF CARTILAGE

Neethu Mohan, Dexy Joseph, Geetha K, Prabha D. Nair
Division of Tissue Engineering & Regenerative Technologies, Sree Chitra Tirunal Institute for Medica sciences & Technology, Trivandrum, Kerala, India

Objective: The study investigates the potential of encapsulating chondroitin sulfate (CS) in fibers of electro spun membranes for recreating an ideal microenvironment for stem cell differentiation to chondrocytes and as raw material delivery scaffold for faster synthesis of extra cellular matrix.
Methods: Adipose derived stem cells (ADMSC) were isolated from adipose tissue of 6 months old New Zealand white rabbits with the approval of institutional animal ethical committee. The cells were characterized for the stem cell markers using flow cytometry. The cells from passage 2 were seeded at a density of 1x 10^6 cells/ml on electrospun poly(caprolactone) (PCL) membrane, PCL membrane encapsulated with 1 wt% chondroitin sulfate (PCL-CS 1) and with 10 wt% of chondroitin sulfate (PCL-CS 10). The cells were cultured for a period of 7, 14, 21 days in two different conditions, namely αMEM with 5% FBS without any chondrogenic induction and in culture medium supplemented with transforming growth factor beta-3 (TGFβ-3) for initial 7 days. At the endpoints, the constructs were characterized for chondrogenesis using biochemical assays, histology, immunohistochemistry and gene expression studies.

Results: The Scanning electron microscopy (SEM) images and atomic force microscopy (AFM) data showed that there was nearly uniform distribution of CS in individual fibers of the electrospun membranes. The Fourier transform IR spectroscopy (FTIR) of the CS released from the fibers indicated that the membranes served as a delivery vehicle for chondroitin sulfate and the chemical structure of released CS was unchanged. Both the entrapped and released CS were taken up by the cells and used as raw materials for faster synthesis of extra cellular matrix. CS encapsulation influenced the ADMSC spreading. The biochemical analysis and histology staining for collagen showed that membranes encapsulated with CS at high dose outperformed the other constructs in total collagen content. The synthetic PCL membrane promoted the synthesis of new GAGs and aggrecan without any chondrogenic induction and raw material supplements. Chondroitin sulfate at both low and high dose did not produce a significant increase in GAG content. The immunostaining and the gene expression data showed however with an initial chondrogenic induction of TGFβ-3, the PCL-CS membranes at both the concentrations outperformed the synthetic PCL in chondrogenesis.
Conclusion: Encapsulating chondroitin sulfate in the matrix has promising approach in stem cell spreading, differentiation to chondrocytes and in *in vitro* engineering of cartilage. They have the potential to significantly enhance the secretion and build up of new extra cellular matrix in 3D scaffolds. Encapsulating raw material like CS is a commercially viable approach to reduce or partially replace the expensive growth factor supplements for faster *in vitro* engineering of cartilage.

F-3063

AN EFFICIENT METHOD TO DIFFERENTIATE CORD BLOOD DERIVED MESENCHYMAL STEM CELLS INTO CHONDROGENIC LINEAGE

Naimisha Reddy Beeravolu, Christina McKee, Sumi Dinda, Rasul Chaudhry
Oakland University, Rochester, MI, United States

Stem cell based regenerative therapies have gained growing attention recently. Human umbilical cord blood derived stem cells have more primitive and promising populations of multipotent stem cells compared to bone marrow derived stem cells. The differentiation potential of human umbilical cord blood derived mesenchymal stem cells (CB-MSCs) is not fully elucidated yet and has been a challenging task. It is also known that CB-MSCs take longer to differentiate into any specific lineage compared to bone marrow derived mesenchymal stem cells (BM-MSCs). The goal of this study was to understand the potential and efficiency of CB-MSCs to differentiate into chondroprogenitors capable of yielding cells that produce extracellular matrix, glycosaminoglycans (GAGs). Tissues such as cartilage and nucleus pulposus in the intervertebral disc are rich in GAG content that are produced by chondrogenic cells but are difficult to regenerate. CB-MSCs expressing mainly CD44 ($CD34^-$) were made into spheroids at a concentration of $1X10^5$ and induced with defined chondrogenic differentiation medium for five days in eppendorfs followed by their transfer onto culture plate for a differentiation period of three weeks. Chondrogenic differentiation was evaluated by analyzing accumulation of GAGs using alcian blue staining, quantitative polymerase chain reaction (qPCR) and immunohistological analysis for chondrogenic specific markers. Plating of spheroids allowed almost uniform differentiation of CB-MSCs as an indicative of cell morphology and production of GAG. Furthermore, the differentiated CB-MSCs expressed chondrogenic markers such as Aggrecan, Sox9, Collagen 2, and Galectin 3. This study demonstrates that we could efficiently differentiate CB-MSCs into chondrogenic derivatives by beginning with very less cell concentration,without requiring longer differentiation period to express extracellular matrix and ending up with a higher yield of differentiated cells.This also verifies that this method of culturing promoted higher chondrogenic differentiation than the monolayer culture or micro-mass culture of CB-MSCs by providing an efficient and optimized differentiation conditions.Therefore, CB-MSCs can be used as a gold standard cell source for autologous or allogenic grafts to treat cartilage disorders, injuries and musculoskeletal regenerative therapies. However, *in vivo* studies will be required to substantiate our *in vitro* findings.

F-3064

IDENTIFICATION OF PUTATIVE STEM CELLS IN PRIMARY CULTURES DERIVED FROM RAT MOLAR PERIODONTAL LIGAMENT AND SUBCULTURES OF MYOGENIC-LINEAGE COMMITTED CELLS

Taka Nakahara[1], Noriko Tominaga[1], Hiroshi Ishikawa[2]

[A]Department of Developmental and Regenerative Dentistry, [B]Department of NDU Life Sciences, [1]The Nippon Dental University School of life Dentistry at Tokyo, Tokyo, Japan

Introduction:

Periodontal ligament (PDL) is a connective tissue consisting of several cell types *in vivo*, and PDL-derived primary cultures are heterogeneous *in vitro*. We have recently established a new method, based on filter paper, to isolate a target cell population from rat PDL primary cultures. Using this method, we isolated cells from primary cultures and characterized them as a skeletal muscle cell population. In the present study, we hypothesized that the skeletal muscle cells emerging from the primary PDL cultures originate from putative stem cells with pluripotency and high plasticity, since skeletal muscle cells are not found in PDL *in vivo*. We aimed to identify the origin of the myogenic cells and to evaluate the putative stem cells in subsequent cultures.

Materials and methods:

Extracted molars from 6-week-old male SD rats were placed in 60-mm culture dishes (2-3 teeth per dish) and were cultured in DMEM/F12 supplemented with 15% fetal bovine serum (FBS). Within 1.5 months after beginning whole tooth cultivation, fibroblastic and epithelial cells grew out from the PDL surrounding the tooth root and reached confluency. We found a morphologically distinct cell population with high cytoplasm to nuclear ratio and some multinucleation among the fibroblastic cells. To isolate the cell population, we performed a method called “cell fishing” (Tominaga et al. *Differentiation*, 2013, in press). The isolated myogenic cells were subcultured 1:3 after reaching confluency. To identify the putative immature stem cells, RT-PCR and immunocytochemistry were carried out for the pluripotent cell markers *Nanog*, *Oct4* and *Sox2* at primary culture and at passage 4. To induce osteogenic differentiation, the subcultured myogenic cells were maintained in osteogenic inductive medium for 3 weeks.

Results:

In the confluent primary PDL cultures, the mono/multinuclear cell population with high cytoplasm to nuclear ratio was observed among the fibroblastic cells and was subjected to RT-PCR and immunocytochemistry. RT-PCR detected the three pluripotent cell marker genes, and immunocytochemistry additionally showed a positive reaction for the antibody against Oct4. The subcultured myogenic cells failed to form alizarin red-positive mineralized nodules, while the subcultured PDL cells, which had been passaged by conventional trypsin/EDTA treatment, extensively formed the characteristic nodules after 3 weeks of osteogenic induction.

Discussion:

Based on the immunocytochemistry and RT-PCR results, primary PDL cultures contain a putative PDL stem cell compartment expressing pluripotent marker genes, suggesting that the skeletal muscle cells arise from immature stem cells among the primary PDL cells. The isolated and subcultured myogenic cells did not form mineralized nodules under osteogenic culture conditions, suggesting that the subcultured cells do not contain the putative stem cells capable of forming the mineralized nodules; on the other hand, the population consists of a myogenic lineage-committed cell population comprising myoblasts and myotubes, because the lineage-committed myogenic cells would not form the mineralized nodules. Collectively, the present cell culture protocol, which requires no chemicals to induce myogenic differentiation, is expected to provide a physiological approach to the handling of somatic stem cells.

F-3065

C-KIT/STEM CELL FACTOR (SCF) SIGNALING CONTROLS PROLIFERATION AND DIFFERENTIATION OF HUMAN MESENCHYMAL STROMAL CELLS (HMSCS)

Supreda Suphanantachat[1], Takanori Iwata[2], Jun Ishihara[4], Masayuki Yamato[3], Teruo Okano[3], Yuichi Izumi[5]

[1]Periodontology, Tokyo Medical and Dental University, Institute of Advanced Biomedical Engineering and Science, Tokyo Women’s Medical University, Tokyo, Japan, [A]Oral and Maxillofacial Surgery, [2]Institute of Advanced Biomed-

ical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan, [3]Division of Cellular Therapy, The University of Tokyo, Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan, [4]Periodontology, Tokyo Medical and Dental University, Tokyo, Japan

C-kit/Stem cell factor (SCF) signaling activates several functions in various types of cells, including cell proliferation, migration, differentiation, and apoptosis. Despite a low number of c-kit positive population found in human mesenchymal stromal cells (hMSCs), the significance of c-kit positive cells has been raised into the field of regenerative medicine. Therefore, the purpose of this study was to investigate the existence of c-kit positive fraction and the role of c-kit/SCF signaling in periodontal ligament-derived hMSCs. Fluorescence-activated cell sorting analysis showed c-kit positive populations (0.65 ± 0.3%, n=7) were found in hMSCs. Real-time PCR confirmed the gene expression of c-kit in hMSCs. The colony forming ability of c-kit positive hMSCs was significantly higher than those of the unsorted cells. After being stimulated with 10 ng/mL of recombinant human SCF, the proliferative activity of hMSCs increased. The gene expression of c-kit in hMSCs was down-regulated after a 24-hour stimulation with various concentrations of cytokines and growth factors related to proliferative activity. Platelet-derived growth factor-BB (0.1 to 20 ng/mL) decreased the gene expression of c-kit dose-dependently. Certain dosages of fibroblast growth factor-2 (10 ng/mL), transforming growth factor-β1 (0.1-500 ng/mL), and enamel matrix derivatives (Emdogain?) (50-100 μg/mL) also strongly suppressed the level of c-kit gene expression. In contrast, stimulation with 500 ng/mL bone morphogenic proteins (BMPs), including BMP-2, BMP-6, and BMP-2/6 heterodimers that have been regarded as cytokines involving in osteogenic differentiation of hMSCs, enhanced 2-4 fold up-regulation of c-kit gene expression. These results suggested that c-kit positive population existed in hMSCs and c-kit/SCF signaling had an important role in controlling stem cell activities in terms of the proliferation and differentiation of hMSCs.

F-3066

HUMAN CRANIAL BONE DERIVED STEM CELLS HAVE MULTIPOTENCY AND THE REMARKABLE CAPACITY OF NEURAL DIFFERENTIATION

Hiroyuki Uwatoko[1], Takafumi Mitsuhara[2], Takuro Magaki[3], Yunosuke Okura[4], Kyousuke Nakata[1], Masaya Matsumoto[1], Takahiro Fukazawa[1], Takeshi Imura[1], Ya-Nan Sun[1], Takuma Furukawa[4], Kawahara Yumi[5], Kaoru Kurisu[2], Yuge Louis[1]

[1]Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan, [2]Department of Neurosurgery, graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan, [3]Department of Neurosurgery, Hiroshima Prefectual Hospital, Hiroshima, Japan, [4]Institute of Health Sciences, Hiroshima University, Hiroshima, Japan, [5]Space Bio-Laboratories Co. Ltd., Hiroshima, Japan

In the last decade,a variety of cell types,including human neural stem cells,embryonic stem cell derivatives,and adult bone marrow stromal cells have been investigated the therapeutic benefits for particular neurological disorders.Bone marrow stromal cells (BMSCs)have suggested having the capacity to differentiate into neural lineages and to be clinically attractive because autologous transplantation can be performed in humans.Although most studies with BMSCs have used those originated from iliac bone, a few reports have dealt with the BMSCs originated from different tissues like tooth-derived stem cells or adipose-derived stem cells,and demonstrated their unique cellular characteristics for the neuro-regenerative activities.Human cranial bones are thought to originate from the cranial neural crest that plays important role for constructing central nervous systems in the embryo.We previously reported that human cranial bone marrow stromal cells(HCBMSCs)had advantage on neural differentiation but didn't show the multipotency.Here,we have investigated HCBMSCs capacities of mesodermal differentiation.If it is shown that HCBMSCs has multipotency,it will become a powerful source of regeneration medicine.Bone marrow samples were obtained from the volunteer's fronto-temporal cranial bone wastes in neurosurgical procedure after informed consent according to the hospital's guidelines.The Bone marrow samples harvested culture dishes and the medium was exchanged to eliminate floating bone powder and non-adherent cells.Cells were exchanged fresh medium in twice a week and incubated until 90% confluence.The cells adhered to the bottom of the culture dish

were used as HCBMSCs.Flow cytometry analysis showed that HCBMSCs expressed a set of mesenchymal stem cell markers.Subsequently,the cells were induced to differentiate into osteocyte,chondrocyte,and adipocyte with each appropriate cytokine combinations.After differentiation,RT-PCR and histological staining demonstrated that mesodermal differentiation from HCBMSCs expressed marker for osteocyte,chondrocyte,and adipocyte. In this study,we show that HCBMSCs has potency to differentiate into mesodermal cells *in vitro*,and would be a novel alternative source of autologous adult stem cells for cell based therapy.

F-3067

PLURIPOTENCY IN STEM CELLS DERIVED FROM MOUSE AND HUMAN WHITE MATURE ADIPOCYTES

Medet Jumabay, Raushan Abdmaulen, Albert Ly, Kristina I. Boström
Medicine, University of California, Los Angeles, Los Angeles, CA, United States

White mature adipocytes give rise to so-called de-differentiated fat (DFAT) cells that undergo multilineage differentiation. The objective of this study was to define stem cell characteristics during DFAT cell generation as a function of time after adipocyte isolation, and their relationship to lineage differentiation. In this study we provide new method for isolation, preparation and maintenance of pluripotancy DFAT cells. Furthermore, we compared pluripotancy of DFAT cells with embryonic stem cell and adipose tissue stromal cells. Both mouse and human DFAT cells, prepared from adipose tissue and lipoaspirate, respectively, showed evidence of pluripotency, with 5 days after adipocyte dedifferentiation. The DFAT cells spontaneously formed clusters in culture, which expressed multiple stem cell markers in a transient fashion, including stage-specific embryonic antigen (SSEA)-1 and -3, Oct3/4, SOX2, Klf4, c-Myc, and the mesenchymal stem cell markers Sca-1 (mouse) and CD105 (human), as determined by real-time PCR, fluorescence activated cell sorting, and immunostaining. As the stem cell markers decreased, the cells spontaneously expressed markers of lineages characteristic of the three germ layers including alpha-fetoprotein (endoderm), neurofilament (ectoderm), and troponin I (mesoderm). However, unlike ES cells, pluripotent DFAT cells do not form terotomas in lever, kidney and testes of immune-deficient mice. A novel modification of the adipocyte isolation aimed at ensuring the initial purity of the adipocytes significantly enhanced the stem cell characteristics in the DFAT cells. Thus, adipocyte-derived DFAT cells spontaneously exhibit pluripotency, which was responsive to changes in culture conditions and may benefit cell-based therapies.

F-3068

EFFECT OF EPIGENETIC ALTERATIONS ON THE DIFFERENTIATION POTENTIAL OF DENTAL PULP STEM CELLS

ECE AKHAN[1], SELİM ERKUT[2], SİBEL YILDIRIM[3], KAMİL CAN AKÇALI[1]
[1]Molecular Biology and Genetics, Bilkent University, Ankara, Turkey, [2]Dentistry Medicine, Baskent University, Ankara, Turkey, [3]Dentistry Medicine, Selcuk University, Konya, Turkey

Dental Pulp Stem Cells (DPSCs) are multipotent mesenchymal progenitor cells with ectomesenchymal origin and have the ability of self renewal and differentiation into multiple lineages including odontoblasts, neural progenitors, osteoblasts, chondrocytes, and adipocytes. These progenitor stem cells are recognized not only as an important source for dentine regeneration but also tissue regeneration and engineering since they are the most accessible source of postnatal stem cells. These cells have higher proliferation potential compare to MSCs and also bear some embryonic stem cells markers. The differentiation of DPSCs into adipocytes is a controversial issue. In this study, we investigated genetic and epigenetic mechanisms to explain the differences of the differentiation of DPSCs into adipocytes. Interestingly, DPSCs which have different differentiation potential for adipogenic differentiation had no difference between their osteogenic differentiations. We analyzed stem cell as well as Epithelial Mesenchymal Transition (EMT) markers on the DPSCs that showed differential adipogenic differentiation potential. Our results did not reveal any difference in the expression of these markers between these cells and therefore we focused on the epigenetic regulations such as histone modifications and CpG methylation patterns at the promoter region of adipogenic lineage specific genes. We examined the expression of H3K4me, H3K4me3, H3K9me2, H3K9me3, H3K27me2, H3K27me3, H3K36me2, H3K36me3 as well as methyltransferases and demethylases such as set 7/9,

G9a, EzH2 and JARID1A, JMJD1, JMJD both with western blot and immunofluorescence. We found significant difference in the expression of H3K4me, H3K36me3 and set 7/9, JMJD1, JMJD2 in DPSCs that have different adipogenic differentiation potential. Alterations in histon modifications along with CpG methylation patterns at the promoter regions of ppar gamma and lpl suggest the involvement of epigenetic regulations on the differentiation potential of DPSCs. In conclusion, the differentiation of DPSCs can be modulated by the factors that affect epigenetic alterations and can therefore be useful in directing the differentiation of DPSCs and may help to develop new clinical treatment modalities in regenerative medicine and dentistry.

F-3071

OBTENTION OF MESENCHYMAL STEM CELLS FROM CRYOPRESERVED UNITS OF UMBILICAL CORD BLOOD

Luz Mabel Avila Portillo[1], Faisury Reyes[2], Derly franco[1], Jenniffer Priscila Avila[1], Sebastian Robledo[3], Angela Riveros[1], Jose Vicente Carmona[4]

[1]Regenerative Medicine Stem Lab, Bogota, Colombia, [2]Universidad Catolica de Manizales, Bogota, Colombia, [3]Universidad de los Andes, Bogota, Colombia, [4]Hospital Militar Central, Bogota, Colombia

Introduction

Approximately 500.000 units of UCB are cryopreserved in public and
private banks all around the world. In the last decade, it has been shown an increasing of tendency to implement theses technologies to many controlled clinical essays in regenerative medicine. The objective of this project is to isolate and culture mesenchymal stem cells from cryopreserved units of umbilical cord blood cultivated with lysate platelet rich plasma.

Design and methods: All samples were obtained with informed consent, the collection of the UCB samples was carried out by the Gynecology Service of Hospital Militar Central; the UCB were processed by volume reduction, automatically frozen and stored at -196 °C for 1 year. Later
they were thawed using PBS, HESSICO and ACDA 1%, LPRP 10%. To obtain the mononuclear cells, used density gradient, count and cellular viability. the culture were plated at a density of $1x10^6$ CMN/cm^2 in DMEM LG supplemented with LPRP, the caracterization by flow cytometry was realized with CD34, CD45, CD73, CD90, CD105 and osteogenic differentiation potential was measure.

Results

Analyzing the results, a volume average of 75.17 ml is observed, a
range of 40 ml - 151.26 ml, the recount of mononuclear cells (CMN) has an average of $9,36x10^8$ CMM and a range of $5,32x10^8$-$1,69x10^9$ the results of CD34 + cells and average of $3,4x10^6$ and a range of $2,49x10^5$ - $15x10^6$, the percentage of CD34 of 0,23 and range of 0,06 - 0.50 and cellular viability of 98.66%, 5/12 of the samples are thawed with a recovery CMN of 95% and average viability of 90%. The 5 cultivated samples stared to show adherence after 8 days of culture, reaching an optimum confluence at day 15, 3/5 of all of them (60%) showed osteogenic differentiation when tintion with silver nitrate (Von Kossa), alkaline phosphatase was performed and positive phenotype for CD90, CD105, CD73 and negative for CD45 and CD34.

Discussion

This project showed the presence of mesenchymal stem cells in 3/5 (60%)
cryopreserved blood units of processed umbilical cord blood with DMSO/Dextran and stored at -196 °C.

F-3072

HGF SECRETING INDUCED PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL PROGENITORS (HIPSC-MPS) MAKE MMPS/TIMPS ORCHESTRA IN ACUTE LIVER FAILURE MODEL FOLLOWING TRANSPLANTATION

Mohsen Moslem, Hossein Baharvand, Mojtaba Rezazadeh Valojerdi
Royan Institute, Tehran, Iran, Islamic Republic of

Objective: Encephalopathy exhibited in Acute Liver Failure (ALF) has been recognized as the responsible for the lethality in this failure. Matrix metaloproteinases (MMPs) and their tissue inhibitors (TIMPs) have significant role in progress of brain edema in ALF. Mesenchymal stem cells (MSCs) therapy can be an alternative treatment for liver transplantation, the only effective treatment for ALF. Our aim was to characterize hiPSC-MPs and evaluate MMPs/TIMPs expression in ALF model following cell transplantation.
Materials and methods: Spontaneously differentiated cells around human iPS cell colonies were mechanically isolated and cultured in MSC medium. passage 4 hiPSC-MPs were characterized by MSCs gold standard characterization methods. hiPSC-MPs and human bone marrow mesenchymal stem cells (hBM-MSCs) were transplanted into ALF model. Animals survival rate, cell integration in liver, in vitro secretion and in vivo expression of Hepatocyte Growth Factor and expression of Collagen type I, MMP13, MMP9, MMP2, TIMP1 and TIMP 2 were evaluated in day 1, 3, 6 and 12 post transplantation.
Results: Our results indicated that mechanically isolated hiPSC-MPs resemble MSCs in morphology, surface markers and differentiation capabilities. In vitro secretion investigation demonstrated significantly higher secretion of HGF in BM-MSCs and iPSC-MPs compared to human fibroblasts Transplantation of hiPSC-MPs into ALF mice model revealed that they could rescue animals from lethality and integrated cells within the liver also significantly decrease collagen type I, MMP13, MMP9 gene expression in liver tissue post transplantation and also increase TIMP1 and TIMP2 expression.
Conclusion: We conclude that hiPSC-MPs can be an unlimited and patient-specific cell source that resemble hBM-MSCs and they can ameliorate MMPs/TIMPs imbalance in ALF model via paracrine secretion of hepatotrophic factors such as HGF.

F-3073

OBCADHERIN REGULATES MSC DIFFERENTIATION INTO SMOOTH MUSCLE CELLS AND DEVELOPMENT OF CONTRACTILE FUNCTION IN VIVO

Stella Alimperti[1], Hui You[1], Sandeep Agarwal[2], Stelios Andreadis[1]
[1]Chemical and Biological Engineering, SUNY at Buffalo, Buffalo, NY, United States, [2]Department of Medicine, Baylor College of Medicine, Houston, TX, United States

Differentiation of mesenchymal stem cells (MSC) is affected by many soluble and insoluble signals in their local microenvironment. Here we report that increasing cell density induced expression of myogenic genes and development of contractile phenotype in MSC even in the absence of soluble differentiation-inducing factors. OB-cadherin was necessary to mediate MSC differentiation into the myogenic fate through activation of key myogenic regulators such as serum response factor (SRF) via the ROCK pathway. OB-cadherin also regulated expression of TGF-β1, which further enhanced myogenesis via the Smad2/3 pathway. However, myogenic differentiation proceeded even in the absence of Smad2 or the TGF-β receptor II, suggesting that the TGF-β1 pathway was not required to promote myogenesis in the presence of intercellular adhesion signaling. Similarly, OB-cadherin also regulated expression of N-cadherin, which was not required for myogenic differentiation. Finally, engagement of OB-cadherin increased its own expression through ROCK1/2 and SRF, suggesting the presence of an autoregulatory, self-sustaining feedback loop that committed MSC to myogenic fate through direct and indirect pathways. Notably, SMC-containing tissues such as aorta and bladder of OB-cadherin knockout mice, showed significantly reduced levels of αSMA and MYH11 and exhibited diminished contractility in response to receptor and non-receptor mediated agonists. These results demonstrate that intercellular adhesion through OB-cadherin activates multiple signaling pathways that initiate and sustain commitment of MSC to myogenic lineage in vitro and in vivo.

F-3074

BIOLOGICAL CHARACTERISTICS OF FELINE ADIPOSE-TISSUE-DERIVED MESENCHYMAL STEM CELLS ISOLATED FROM VISCERAL AND SUBCUTANEOUS ADIPOSE TISSUE

Qian Qin[1], C Earle Pope[1], Guoshun Wang[2], Robert A. MacLean[1], Martha C. Gomez[1]

[1]Audubon Center for Research of Endangered Species, New Orleans, LA, United States, [2]Louisiana State University Health Sciences Center, New Orleans, LA, United States

Adipose-tissue-derived mesenchymal stem cells (AMSCs) are a valuable cell source of regenerative medicine for injured and geriatric animals, including those from endangered species. In the domestic cat, fAMSCshave been isolated from subcutaneous (SQ) adipose tissue (*Santos et al., 2012, ISSCR pp 216*). Whether AMSCs isolated from visceral fat of the abdominal cavity (AB) have similar developmental potential has not been studied. In this study, we compared the biological characteristics of domestic cat AMSCs isolated from AB and SQ adipose tissue. Adipose tissues were harvested via abdominal laparoscopy and digested with collagenase II at 37 ºC for 30 min. Cells from the stromal vascular fraction were cultured in DMEM-F12 medium with 12% fetal bovine serum under 5% CO_2 at 37 ºC. Results showed that after 48-72 h, AB- and SQ-fAMSCs attached, assumed a fibroblastic morphology, and formed colonies. AB- fAMSCs showed a higher proliferation capacity (1.6 days) than that of SQ- (3.8 days) by counting cell doublings. Flow cytometry analysis revealed higher percentages of $CD90^+$(85%) and $CD105^+$(82%) cells in AB-fAMSCs than in SQ- fAMSCs(71%, 58%; respectively). Only AB-fAMSCs were $CD146^+$(35%) and $CD271^+$(4%). Both AB and SQ-AMSCs contained small subpopulations of HLA-DR^+ cells (9%, 7%, respectively) and showed negative expressions of $CD14^-$, $CD45^-$ and $CD73^-$. Moreover, both AB- and SQ-fAMSCs demonstrated differentiation potential toward adipogenic and neurogenic lineages. Our preliminary results suggest differences (e.g.morphological) in the extent of differentiation between these two cell groups. The biological significance of these differences requires further investigation. We concluded that AMSCs were easily accessible from the domestic cat and differences in cell proliferation and expression of cell surface markers between SQ and AB-fAMSCs were observed. Our intention is to continue the fAMSC study with a goal of eventually realizing therapeutic utility in geriatric or infirmed endangered cats in captivity.

F-3075

HUMAN MESENCHYMAL STEM CELLS SUPPRESSED ANTIGENECITY OF MOUSE PBMNC IN THE RATS VIA INDUCTION OF FOX3 CD25 TREG CELLS

SEONG EUN KOH[1], Young-Jay Lee[2]

[1]Rehabilitation Medicine, Konkuk University Medical Center, Seoul, Korea, Republic of, [2]Maria Biotech Co., Seoul, Korea, Republic of

Presence of mesenchymal stem cells (MSC) were reported from the almost every body parts studies and are believed to be involved in the homeostasis of the organism. Due to this reason, MSCs were considered to be a prime candidate for the cell therapies to replace the damaged or degenerated, thereby, non-functional tissues. Recently, however, transplantation studies revealed that the duration of mouse skin graft to baboon was extended when cord blood MSCs were co-transplanted.

To understand the spectrum of MSCs and the mechanism involved in the immunomodulation, several MSCs were isolated from the human endometrium (EnMSC). The established EnMSCs were able to suppress conconavalin A (con A)-stimulated proliferation of rat peripheral blood mononucleocytes (rPBMNC). A similar suppression of rPBMNC was observed when mitomycin C-exposed mouse PBMNC was used as antigen in the mixed lymphocyte reaction experiments. Flow cytometric analyses confirmed that the ratio of FoxP3+ CD25+ double positive cells were increased from 1.7% to 6.5% when EnMSCs were present at the expense of CD4+ FoxP3- T cells (from 22.9% to 8.9%), suggesting the induction of T_{reg} cells and the suppression of T_{eff}. Furthermore, the number of rats and the amount of anti-mouse IgG and IgM produced by the rats were drastically suppressed when EnMSC was co-transplanted with mitomycin C-exposed mouse PBMNC. RT-PCR confirmed the involvement of EnMSC in the immunosuppression: IL-1β being suppressed and IL-10 and TGF-β being induced. Taken together, human EnMSC, although extensive studies are needed, may be considered as a promising candidate to replace the toxic pharmacological immunosuppressants in the clinical transplantations.

F-3076

ENDOGENOUS PRODUCTION OF INDIAN HEDGEHOG IS ESSENTIAL FOR HUMAN MESENCHYMAL STEM CELL CHONDROGENESIS

Andrew M. Handorf, Wan-Ju Li
Department of Orthopedics, University of Wisconsin-Madison, Madison, WI, United States

Introduction

Human mesenchymal stem cells (hMSCs) hold great promise in articular cartilage tissue engineering applications, given that they have immunosuppressive properties and can be readily driven into the chondrogenic lineage upon exposure to TGFβ ligands. However, hMSC-driven articular cartilage formation is currently limited by the production of hypertrophic tissue that ultimately forms bone when implanted subcutaneously *in vivo* through a process reminiscent of endochondral ossification. Thus, a better understanding of the molecular mechanisms driving hypertrophic differentiation is needed if hMSCs are to have clinical utility in treating degenerative joint diseases like osteoarthritis.

During development, induction of chondrocyte hypertrophy has been shown to be promoted, at least in part, by the growth factor, Indian Hedgehog (IHH). Here, we examined whether IHH plays a similar role in promoting hypertrophy during hMSC chondrogenesis, with the overall goal of producing phenotypically stable articular chondrocytes devoid of hypertrophy.

Methods

Human bone marrow-derived MSCs were expanded for two passages in DMEM-LG containing 10% FBS and 5 ng/mL FGF2. Upon 70% confluence, hMSCs were collected for chondrogenic differentiation in pellet culture via induction with 10 ng/mL TGFβ1 in standard chondrogenic medium.

Results

We began by analyzing the expression of *Ihh* every three days throughout hMSC chondrogenesis in pellet culture to determine if differentiating hMSCs are producing IHH. In so doing, we found that *Ihh* was expressed throughout differentiation and showed a dramatic 6-fold peak in expression from Days 9-12 of differentiation in pellet culture. Interestingly, this coincided with a dramatic increase in the expression of a variety of hypertrophy-associated genes, including *collagen type X* (*Col X*), suggesting that perhaps the endogenous production of IHH is promoting hypertrophy in hMSC-derived chondrocytes. In support of this hypothesis, there was a very strong correlation ($R^2 > .99$) between the expression of *Ihh* and *Col X* at all time points tested regardless of the treatment employed. Further, we found that exogenous TGFβ1 exposure to differentiating hMSCs induced a consistent and sustained 9-fold increase in *Ihh* expression within six hours, suggesting that perhaps our current differentiation conditions are inadvertently inducing hypertrophy through the endogenous production of IHH.

To test whether the endogenous production of IHH is indeed promoting chondrocyte hypertrophy, we used a pharmacological inhibitor of Hedgehog signaling_cyclopamine_and shRNA to knockdown *Ihh* expression. Using both methods, we found that inhibition of IHH

almost entirely blocked the induction of hMSC chondrogenesis instead of
hypertrophy specifically, as evidenced by a blockade in *Col II* gene expression and absence of Col II immunofluorescence
staining within pellets. Further, there was a marked increase in apoptosis in *Ihh* knockdown cells. Taken together, these data demonstrate that endogenous IHH is essential for the successful
execution of hMSC chondrogenesis and cell survival.

Conclusions

1.
There is a strong correlation between *Ihh*
and *Col X* expression throughout
differentiation.
2.
Exogenous TGFβ1 treatment induces a sustained increase in *Ihh* expression within six hours of exposure.
3.
Endogenous IHH production is necessary for TGFβ1-mediated induction of hMSC
chondrogenesis and is important for cell survival.

F-3077

FETAL LIVER MESENCHYMAL STEM CELLS- DERIVED DOPAMINERGIC NEURONS FOR THERAPEUTIC CORRECTION OF PARKINSONS'S DISEASE IN MOUSE MODEL

Amit Kumar
Stem Cell Biology Lab, National Institute Of Immunology, New Delhi, India

The possibility for use of stem cells as therapeutics has generated tremendous opportunities among the researchers for exploring cells from diverse tissue sources. Various studies in primate and non-primate models have shown exciting results in terms of functional recovery and ablation of the disease symptoms. Different kinds of stem cells, as on today, being used for the therapy are embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs) and neural stem cells (NSCs).
In the present study we have tried to identify a new source of MSCs-like cells and isolate them from the liver of developing embryo. These cells are believed to be more primitive and hence may have superior differentiation potential. We have sorted these fetal liver cells (FL-MSCs) for CD45- Ter119- CD44+ phenotype and characterized them by flow cytometry with respect to the various known MSC markers, such as CD73, CD29, CD105, CD49e and for the hematopoietic markers like CD14, CD40, CD80, CD86. We further studied gene expression profile of these cells for MSC and other lineages. These cells were differentiated into osteogenic, adipogenic and neuronal dopaminergic (DA) lineage. Characterization of the DA neurons was done by reverse transcriptase polymerase chain reaction (RT-PCR) and immunocytochemistry (ICC) for TH, microtubule associated protein 2 (Map2), dopamine transporter (DAT), β-tubulin III (Tuj1), NeuN, 5HT receptor, and glial fibirllary acidic protein (GFAP). Functional characterization of the differentiated cells was conducted using whole - cell patch - clamp technique. We then transplanted these differentiated cells into 6-OHDA-induced Parkinson's disease (PD) mouse model and analysed for the functional recovery.
Our results suggest that, FL-MSCs are phenotypically resembles to adult bone marrow-derived MSCs and uncommitted to the target population. Differentiation of FL-MSCs into adipogenic, osteogenic and neuronal lineage suggests their multipotent behaviour. Differentiated neuron like cells showed the expression of various dopaminergic markers. Functional characterization by whole - cell patch -clamp recording also showed fast inactivating of K+ currents, which can be blocked by 4APD, implying that the in vitro transdifferentiated cells are functional neurons. Transplantation of cells into PD mouse showed engraftment of the cells followed by recovery in motor functional activity. Thus, our results propose an alternate source of cells that can be used for the therapy of PD in mouse model.

F-3078

EXTRACELLULAR MATRIX PROTEIN LAMININ INDUCE ENDOTHELIAL POTENTIAL OF BONE-DERIVED HUMAN MESENCHYMAL STEM CELLS (MSCS) THROUGH FOXC2 AND αVβ3 INTEGRIN/CD61 AXIS

Chih-Hsiang Wang[1], Ting-Ming Wang[2], Tai-Horng Young[3], Yiu-Kay Lai[4], **B. Linju Yen**[1], Men-Luh Yen[5]
[1]Institute of Cellular & System Medicine, National Health Research Institutes, Miaoli County, Taiwan, [2]Department of Orthopedic Surgery, National Taiwan University Hospital, Taipei, Taiwan, [3]Institute of Biomedical Engineering, College of Medicine, National Taiwan University, Taipei, Taiwan, [4]Institute of Biotechnology, National Tsing Hua University, Hsinchu, Taiwan, [5]Department of Primary Care Medicine & Department of Obstetrics/Gynecology, National Taiwan University Hospital & College of Medicine, National Taiwan University, Taipei, Taiwan

Interactions between blood vessels and osteoblasts--bone-forming cells--are critical for successful bone development. We therefore investigated the endothelial differentiation capacity of mesenchymal stem cells (MSCs) derived from bone tissue. We found that fetal pre-osteoblast and adult trabecular bone-derived (TB) MSCs express similar surface markers as bone marrow (BM) MSCs and can differentiate into adipocytes, osteoblasts, and chondrocytes. However, when cultured in extracellular matrix (ECM) and endothelial differentiation conditions, bone-derived MSCs (B-MSCs) more readily form tubular structures and uptake acetylated low-density lipoproteins, fulfilling the functional criteria for endothelial cells (ECs). Moreover, addition of B-MSCs but not other cells significantly enhanced vessel formation in the in vivo chick chorioallantoic membrane assay. Mechanistically, this appears to be due to the upregulation of the endothelial transcription factor forkhead box protein C2 (FOXC2) and its downstream gene αvβ3 integrin/CD61in B-MSCs but not BMMSCs by laminin, a component protein of the ECM. Taken together, our findings not only reveal discrepant differentiation capacity for various tissue-specific MSCs, but also highlight the critical role of the niche--in this case, the ECM and its component proteins--in determining lineage commitment of stem cells.

F-3081

A XENO-FREE, SERUM-FREE DEFINED MEDIUM TO RAPIDLY DIFFERENTIATE HUMAN MESENCHYMAL STEM CELLS TO OSTEOBLASTS

Anna Abai[1], Jey Jeyakumar[2], Lilian Hook[2], Nick Asbrock[1], **Vi Chu**[1]
[1]Stem Cell, EMD Millipore, Temecula, CA, United States, [2]The London BioScience Innovation Centre, Plasticell Ltd, London, United Kingdom

Mesenchymal stem cells (MSCs) are multipotent adult stem cells that have been traditionally isolated from bone marrow but have now been derived from adipose and placental tissue, and umbilical cord blood and can be generated in vitro from embryonic and induced pluripotent stem (iPS) cells. They harbour the capacity to differentiate into bone, cartilage, and fat cells and are the subject of more than 120 clinical trials for a range of regenerative and inflammatory diseases. The considerable therapeutic potential of MSCs is hampered by current difficulties in directing the differentiation to a single cell-type specific lineage at sufficient high purity and functionality. Current protocols for osteogenic differentiation employ animal-derived serum, a source of lot-to-lot variations and a cause of concern for researchers contemplating a transition to clinical applications. These differentiation protocols require a long time frame, typically 2-3 weeks before the appearance of mineralization and the degree of differentiation is generally dependent upon the tissue source and intra-clonal variations of the stem cell lines derived.
Here we describe development of a defined xeno-free, serum-free osteogenic differentiation medium that can efficiently generate mineralized cultures in under 7 days and works consistently across all sources of MSCs tested, including bone-marrow and adipose tissues and those derived from human embryonic stem cells (hESCs). The formulation of the xeno-free medium was discovered using a high-throughput combinatorial platform, termed Combicult®, which combines miniaturization of cell culture on micro-carriers, a pooling/splitting protocol, and a unique tagging system to allow multiplexing of thousands of experiments in one screen. This approach allowed testing of 3,375 unique differentiation protocols to identify a xeno-free, serum-free medium that promotes the selective differentiation of human MSCs to osteoblasts. A large percentage of mature osteocytes can be generated from all

sources of MSC tested in 7-10 days as opposed to 21 days using standard culture methods. To assess stability, the medium was incubated at 37^{O}C for seven days and underwent two freeze-thaws. Results from the accelerated stability study indicated no loss of activity as compared to the unstressed control and was confirmed with data obtained from a 1 year real time stability study. The stability of the raw material components at 37^{O}C, a temperature frequently used to culture MSCs and a long expiration dating of greater than 2 years at -20^{O}C is expected to help reduce the costs of scale-up manufacture. A reliable, cost effective, and rapid method to produce large amounts of human osteoblasts is an important advance towards wide-spread utilization of stem cells for cell therapy and drug discovery applications.

F-3082

PLURIPOTENCY AND PROLIFERATIVE PROFILE OF RAT BONE MARROW MESENCHYMAL STEM CELLS FROM DIFFERENT AGES

Maria Arufe Gonda[1], Pablo Fernández[1], Isaac Fuentes[1], Javier De Toro[1], Francisco Blanco[2]
[1]Univ of Coruña, A Coruña, Spain, [2]CHUAC, A Coruña, Spain

Mesenchymal stem cells (MSCs) are of highly relevant for regeneration of mesenchymal tissues such as bone and cartilage. The promising role of MSCs in cell-based therapies and tissue engineering appears to be limited due to a decline of their regenerative potential with increasing donor age. We studied the pluripotency and proliferative profile of different bome marrow mesenchymal stem cells -BM-MSCs- from six aging groups of Wistar rat. Groups were; neonate -0 days old-; infant -7 days old-; young -14 days old-; pre-pubertal -20 days old-; pubertal -25 days old- and adult-30 days old. After isolation by plastic adherence of BM-MSCs from femur bones rat, the cells were expanded after 2 passages into RPMI 10% FBS and they were characterized.
Flow cytometry of CD117 and BRDU after labelling was realized. Neonate group show the most significant ($p<0.05$) proliferative incorporating the most percentage of cells labelled with BRDU (45%). However, pubertal and adult were the groups with significant ($p<0.05$) more percentage of CD 117 positive cells in front of the rest of the groups. qRT-PCR analysis of stem cell markers like Rex1, Oct4, Sox2 and Nanog indicated that young group had the most significant increase ($p<0.05$) of Oct4, Sox2 and Nanog genes. However, pre-pubertal group had the most significant increase ($p<0.05$) of Rex1.
Immunohistochemistry analysis was done to study biological differentiation using the specific medium for 14 days to induce ostogenesis, adipogenesis and chondrogenesis. Immunostaining of differentiated cell with Alizarin red to check osteogenesis, Oil red to check adipogenesis, Safranine O and Masson Modified to check chondrogenesis were performed. Densitometry of tissue using the computer program AnalySIS Image Processing (Soft Imaging system GmbH V. 5.0, Olympus, Germany)was evaluated. The results indicated that BM-MSCs pre-pubertal group presented the most significant ($p<0.05$) chondrogenic and adipogenic potential and infant group had the most osteogenic potential.
Taking together these results indicate that mesenchymal stem cells have different pluripotency and proliferative potential depending of animal age, these might be account according with the nature of experiment to realize. So, BM-MSCs from neonate could be used when is necessary to expand the culture in the time and BM-MSCs from pre-pubertal rat for cell therapy to treat pathologies where involving cartilage loss like OA.

F-3083

MITOCHONDRIAL CHANGES DURING CARDIOMYOCYTE AND ADIPOCYTE DIFFERENTIATION OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS: A COMPARATIVE STUDY

Vinod K. Sharma[1], Shalmonali Bhattacharyya[2], Vivekanand Jha[1]
[A]Translational and Regenerative Medicine/Nephrology, [B]Biophysis, [1]Post Graduate institute of medical education and research, Chandigarh, India

Introduction: Progenitor cells are predicted to have higher cellular maintenance activity, active stress defense, low levels of stress generation and lower energy demand to ensure sufficient genomic stability over generation, whereas this would not be necessary for differentiated cells. We tested this hypothesis by examining stress levels (ROS: superoxide and peroxide), mitochondrial mass, cardiolipin content and membrane potential during cardiomyogenic and adipogenic differentiation of bone marrow isolated mesenchymal stem cells (MSCs).
Material and method: MSCs were isolated from 10 ml of human bone marrow by culturing in enriched medium (MEM+10%FBS+Pencillin/streptomycin) in 5% CO2 incubator at 37^{o}C. MSCs were induced to differentiate into cardiomyocyte (5-azacytidine and fibroblast growth factor) and adipocyte (dexamethasone, insulin and indomethacin) over 24 days. The differentiated cardiomyocytes were characterized by staining with cardiac alpha actin, F-actin, myosin and troponin T antibodies by fluorescent microscopy. Differentiated adipocytes were characterized for the accumulation of lipid by staining with Oil Red O. The changes in ROS levels and mitochondrial parameter were recorded as differentiation progresses at Day 1,2,4,8 and 24. To measure cellular superoxide and peroxide levels, cells were incubated with 10µM of dihydroethdine and 5µM of 2',7'-dichlorofluorescin diacetate for 30 mints at 37^{o}C in dark. Mitochondrial mass (MM), cardiolipin content (CL) and mitochondrial membrane potential (MP) were assessed by staining with 50 nM mitotracker green, 50 µm of nonylacridine orange and 5µm 3,3'-dihexyloxacarbocyanine iodide for 20 minutes 37^{o}c in dark. All data were obtained from three independent experiments done with MSCs isolated from three different bone marrow samples and analyzed using flowcytometery and expressed as mean±SD.
Results: During adipogenic and cardiomyogenic differentiation the superoxide levels showed an early decrease (62.38±4.7% and 77.65±1.2% at day 4) and then increased (123.01±4.3% and 197.85±11.5% at day 24). The peroxide levels also decreasedat D4 (61.75±5.6% and 45.49±4.9% at D4) and then increased till D24 (390.32±1.8% and 139.14±5.3%). MM and CL showed a continuous increase till D24 (MM 568.43±17.0% and 731.39±39%; CL 364.64±35.0% and 447.61±38%). MP showed a see-saw effect, with increased at D2 (180.96±3.3% and 155.91±24.8%) followed by a fall at D4 (77.9±18.7% and99.58±14.6%) and a final increase to D24 (267.62±34% and 279.00±25%).
Discussion: The present study show that MSC have lower stress levels (ROS), mitochondrial mass, cardiolipin content and membrane potential. These parameters increase with differentiation to produce functionally differentiated cell type. The lineage specific commitment of MSCs starts with decreased stress levels and increased mitochondrial mass and cardiolipin content, which increases further as MSCs differentiate into mature adipocyte and cardiomyocyte. The higher mitochondrial mass, cardiolipin content and membrane potential in cardiomyocytes compared to adipocytes might signify cell-specific difference in demand.

F-3084

TENASCIN-C AND TENASCIN-R CO-REGULATE NEURONAL DIFFERENTIATION SIGNALING VIA INTEGRIN α7 IN HUMAN MESENCHYMAL STEM CELLS

Hung-Li Tsai[1], Wen-Ta Chiu[4], Chia-Lang Fang[2], Shiaw-Min Hwang[5], Perry Renshaw[6], **Wen-Fu Lai**[3]
[A]Graduate Institute of Medical Sciences, [B]Department of Pathology, [C]Graduate Institute of Clinical Medicine, [1]Taipei Medical University, Taipei, Taiwan, [2]Department of Neurosurgery, Taipei Medical University-Shuan-Ho Hospital, Taipei, Taiwan, [3]Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan, [4]Brain Imaging Center, McLean Hospital/Harvard Medical School, Belmont, MA, United States

Getting human (h)MSCs to transdifferentiate into mature neurons is a major challenge. It was previously found that tenascin-C and -R regulate differentiation, migration, and neurite outgrowth and survival in many types of neurons and progenitors. Hence, we hypothesized that tenascins regulate neurogenic transdifferentiation of bone marrow-derived hMSCs. In a qualitative PCR analysis, we found that adding a tenascin-C and -R mixture to the medium significantly promoted expressions of neuronal and glial markers. When hMSCs were cultured on tenascin-coated surfaces, the qPCR and immunocytochemistry results showed that i) tenascin-C was crucial for neurite outgrowth and synaptic marker expression; ii) tenascin-R promoted neuronal differentiation but inhibited neurite outgrowth and synaptic marker expression; and iii) more significantly, the tenascin mixture promoted differentiation into neurons

and oligodendrocytes, induced neurite and synapse formation, and inhibited differentiation into astrocytes. In a functional blocking study, integrin α7 and α9β1 blocking antibodies both inhibited mRNA expression by hMSCs on the coated tenascin mixture. Taken together, the tenascin mixture significantly promoted hMSC differentiation into mature neurons via integrin α7 and α9β1. This finding will be of great help for stem cell therapy to treat neurodegenerative diseases.

F-3085

HUMAN MESENCHYMAL STEM CELLS/MULTIPOTENT STROMAL CELLS REMAIN IN A STATE OF ARRESTED AUTOPHAGY PRIOR TO DIFFERENTIATION

Austin Nuschke[1], Melanie Rodrigues[1], Donna Stolz[2], Charleen T. Chu[1], Alan Wells[1]
[A]Pathology, [B]Cell Biology and Physiology, [1]University of Pittsburgh, Pittsburgh, PA, United States

Mesenchymal stem cells/multipotent stromal cells (MSCs) have the ability to migrate to injury sites in the body, self-renew, and differentiate into a variety of tissue lineages, including bone, fat, cartilage, and others. Additionally, these cells provide a variety of growth factors that support tissue renewal at sites of injury. As such, MSCs are attractive candidates for use in cell therapies for wound healing, particularly in bone and cartilage lesions. However, MSCs implanted into wound beds are faced with various challenges, including acute nutrient deprivation, hypoxia, ROS generation, and challenge from inflammatory cytokines. Implanted MSCs must therefore not only proliferate in the wound bed, but also survive long enough to differentiate effectively. Our group has previously utilized growth factor tethering to activate survival signaling in MSCs via EGFR in an effort to improve cell viability following post-implantation challenges in the wound bed. However, given that MSCs play a role in normal wound healing, the cells must have an innate mechanism of adapting to this harsh environment. Here, we studied the process of macroautophagy, a mechanism for recycling cellular organelles, hypothesizing that MSCs would utilize this process to adapt to the metabolically challenging conditions of the wound bed.
Using transmission electron microscopy, we found that MSCs grown in physiologic concentrations of glucose (5.5 mM) show a halt in the process of autophagy prior to lysosomal degradation, coinciding with a notable accumulation of autophagosomes in the cell. This accumulation was rapidly decreased when cells were treated with rapamycin to induce autophagy, as well as when cells were cultured in high glucose media (25 mM). To further examine autophagosome turnover, we transfected MSCs with a GFP-RFP plasmid which labels LC3 with both GFP and RFP during early and mid-autophagy, but loses the GFP signal as the lysosome degrades the autophagosome during late autophagy. We found that MSCs in normal culture conditions did not lose GFP signal unless stimulated to undergo autophagy, confirming a cessation of the process prior to autophagosome degradation. We then examined the autophagic response of MSCs when challenged with various types of nutrient deprivation, and found that LC3II expression was high when cells were cultured at physiologic glucose levels, and expression decreased dramatically when switched to 25 mM glucose over a period of four days. Neither oxygen nor serum levels had an effect on LC3 expression. Finally, we saw a loss of LC3II levels as MSCs were differentiated into osteoblasts over 30 days, with faster osteoblast formation at 4% and 1% oxygen tensions.
These results show a novel and unique autophagy phenotype in human MSCs at basal conditions that may be exploited during times of high metabolic demand, such as acute nutrient starvation in a wound site. Ultimately, a carefully balanced autophagic response may be key in MSC survival and differentiation.

F-3086

THE ROLE OF MITOCHONDRIAL BIOGENESIS IN HUMAN MESENCHYMAL STEM CELL DIFFERENTIATION AND ACQUISITION OF A CARDIAC PHENOTYPE

Arun Ajmera[1], Maria C. Collins[1], Payal V. Pradhan[1], Ethan J. Anderson[2], **Barbara J. Muller-Borer**[3]
[A]Cardiovascular Sciences, [B]Pharmacology and Toxicology, [C]Engineering, [1]East Carolina University, Greenville, NC, United States

Introduction: With an increased understanding of myocardial infarction, human mesenchymal stem cell (hMSC) transplantation therapies have shown promising results as a possible treatment for heart failure. However, transplanted stem cells undergo a significant rate of cell loss within 24 hours after transplantation. Furthermore, little is known about mitochondrial biogenesis for hMSCs transplanted to a cardiac microenvironment, even though mitochondria are vital to cell energy production, cellular differentiation, and cell death. The purpose of this study was to investigate the role of mitochondrial biogenesis in adult hMSC differentiation and acquisition of a cardiac phenotype. Based on recent experimental evidence, we hypothesized that mitochondrial activity would be enhanced when hMSC differentiation was directed towards a cardiac-like fate. **Methods:** In this study, two models to direct hMSC differentiation to a cardiac-like phenotype were evaluated. In the 1^{st} model, hMSCs were treated for 2.5 weeks with a combination of 3 growth factors (GF), insulin-like growth factor (2 ng/mL), fibroblast growth factor (50 ng/mL), and bone morphogenetic protein (10 ng/mL). In the 2^{nd} model, hMSCs were co-cultured with neonatal rat cardiac myocytes for 48 hours. For both models qRT-PCR analysis was performed on Mitochondrial Transcription Factor B1(TFB1M), Ubiquinol-Cytochrome C Reductase Core Protein 1 (UQCRC1), Nuclear Respiratory Factor 1 (NRF-1) and Myocyte-specific Enhancer Factor 2C (MEF2C). Mitochondrial and cardiac specific gene expression was compared in control hMSCs (monoculture), GF treated hMSCs and co-cultured hMSCs. For GF treated hMSCs we also evaluated protein expression of N-cadherin, mitochondrial Electron Transport Chain (ETC) complexes II, III, IV and V, and assessed oxidative capacity with mitochondrial O_2 consumption using an Oroboros O_2K Oxygraph system. **Results:** Growth factor treated hMSCs showed increased mitochondrial gene expression of TFB1M ($p=0.04$), increased O_2 consumption, increased protein expression of mitochondrial ETC complex II and an almost 2 fold increase in protein expression of N-cadherin. However, no significant differences in expression of other cardiac specific genes were observed with this model. Co-cultured hMSCs showed increased mitochondrial and cardiac specific gene expression when compared to control for TFB1M, UQCRC1, and MEF2C ($p \leq 0.05$). **Conclusions:** These findings suggest that augmentation of mitochondrial gene expression, content, and oxidative capacity may contribute to hMSC differentiation towards a cardiac-like fate, and suggest that mitochondrial function may be a critical component for enhancing hMSC therapies in cardiac tissue regeneration and repair. Examining the role of mitochondria in hMSC differentiation is critical for developing more effective cell based therapies for cardiac tissue regeneration.

F-3087

PAH/PAA POLYELECTROLYTE MULTILAYER SURFACE EFFECTS ON HUMAN MESENCHYMAL STEM CELL EXTRACELLULAR MATRIX ORGANIZATION AND DIFFERENTIATION INTO OSTEOBLASTS

sudhakara R. pattabhi[1], Ali Lehaf[2], Tom Keller[1], Joseph Schlenoff[2]
[A]Biology, [B]chemistry, [1]FSU, Tallahassee, FL, United States

Biocompatible implant coatings such as hydroxyapatite and polyelectrolyte polymers have been tested to address titanium implant failures, but lack of fixation between implants, coating materials, and tissues remains problematic. In this study, we show that polyelectrolyte multilayers (PEMUs) generated layer by layer with the polycation PAH [poly(allylamine hydrochloride)] and the polyanion PAA [poly(acrylic acid)] and thermally crosslinked to increase surface modulus provide excellent substrates on which human mesenchymal stem cells (hMSCs) can be induced with factors to differentiate into osteoblasts and mineralize the surface. Both PAA-terminated and PAH-terminated PEMUs were assessed. In addition to presenting a different surface charge, the PAA-terminated PEMUs have a greater degree of surface nanotopography than do the smooth PAH-terminated PEMUs. During differentiation into osteoblasts, hMSCs induced on PAA-terminated PEMUs or on glass coverslips expressed higher levels of the early osteoblast differentiation marker alkaline phosphatase sooner than did hMSCs induced on PAH-terminated PEMUs or on polystyrene culture dishes. The cells on the PAA-PEMUs and coverslips also organized Collagen type I, the major ECM protein of bone tissue, into a much more amorphous network than did the cells on PAH-PEMUs, which organized collagen I into larger diameter fibers. Cells induced on all four surfaces progressed to express the later stage osteoblast differentiation markers osteonectin, bone sialoprotein, and osteopontin. By day 18 after induction, however, the cells on the PAA-terminated PEMUs and on the polystyrene culture dishes had deposited more Ca2+-mineralization than had the cells on the PAH-terminated PEMUs or on the glass coverslips. We speculate that the

high modulus, nano featured, negative charge surface properties of thermally-crosslinked PAA-terminated PEMUs may improve osseointegration and implant stability.

F-3088

THE EFFECT OF CALCIUM-INCORPORATED TITANIUM SURFACES ON THE OSTEOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

Rumi Sawada[1], Ken Kono[1], Kazuo Isama[2], Yuji Haishima[1], Atsuko Matsuoka[1]

[A]Division of Medical Devices, [B]Division of Environmental Chemistry, [1]National Institute of Health Sciences, Tokyo, Japan

Human mesenchymal stem cells (hMSCs) are pluripotent and can differentiate into cells of mesodermal origin, e.g., bone, cartilage, adipose, and muscle cells. hMSCs also have the capacity to differentiate into myocytes, hepatocytes, and neural cells. hMSCs are currently being used with biomedical materials in several clinical studies on bone regeneration. Titanium is widely used in orthopedic and dental implants due to its corrosion and wear resistance, durability, and biocompatible interface. The topography of titanium implants plays a major role in cell-material interaction. Surface modifications influence protein adsorption, cell-substrate interactions, cell adhesion, cell morphology, osteogenic differentiation, and, consequently, the tissue integration of titanium implants.
In this study, a titanium surface was chemically modified with calcium ions and assessed for its influence on osteogenic differentiation and molecular responses of hMSCs. We examined the amount of calcium ion incorporation and apatite formation on $CaCl_2$- and $Ca(OH)_2$-treated surfaces. We investigated the morphology, proliferation, and osteogenic differentiation of hMSCs cultured on the chemically modified titanium.
hMSC morphology was altered and its proliferation was significantly reduced by calcium surface treatment of titanium disks. In addition, incorporation of calcium ions and apatite formation on the titanium surface treated with $Ca(OH)_2$ were significantly greater than on $CaCl_2$-treated titanium disks. Structural gene for the bone matrix osteopontin (OPN) transcription in hMSCs was significantly upregulated by the calcium surface treatment, and was significantly on $Ca(OH)_2$-treated disks than on $CaCl_2$-treated disks. The marker of bone formation osteocalcin (OCN) protein expression was upregulated by $Ca(OH)_2$ treatment; however, $CaCl_2$ treatment had no effect on the protein expression of OCN. We conclude that calcium surface treatment of the titanium disks influenced the osteogenic differentiation of hMSCs, and $Ca(OH)_2$ treatment of the titanium surface induced osteogenic differentiation, while $CaCl_2$ treatment had a limited effect. To investigate the mechanism of osteogenic induction in hMSCs by calcium ions, comparative gene expression profiles were assessed by DNA microarray and pathway analyses. We found that $Ca(OH)_2$ treatment significantly upregulated expression of BMP2, PTGS2 (cyclooxygenase 2; Cox2), parathyroid hormone-like hormone (PTHLH), and SPP1 (OPN) in hMSCs. In contrast, Smad signaling was downregulated by chemically modified titanium surfaces. These observations suggest noncanonical BMP signaling (independent of Smad signaling) might mediate the osteogenic differentiation of hMSCs on $Ca(OH)_2$-treated titanium. Furthermore, whole genome expression analysis suggested that calcium modification of the titanium surface activates Wnt/β-catenin signaling.

F-3091

SINGLE INJECTION OF MESENCHYMAL STEM CELLS PROVIDES LONG TERM PROTECTION AGAINST DOPAMINERGIC NEURODEGENERATION IN AN EXPERIMENTAL MODEL OF PARKINSON'S DISEASE

Márcia Cristina L. Pereira[1], Mariane Secco[2], Carolina Oliveira Rodini[3], Laila Brito Torres[1], Bruno Henrique Araujo[1], Esper Abrao Cavalheiro[1], Mayana Zatz[3], Oswaldo Keith Okamoto[3]

[1]Neurologia/neurocirurgia, Universidade Federal de São Paulo, São Paulo, Brazil, [2]Departamento de Genética e Biologia Evolutiva, Universidade Estadual de São Paulo, São Paulo, Brazil, [3]Departamento de Genética e Biologia Evolutiva, Universidade Estadual de São Paulo, São Paulo, Brazil

Mesenchymal stem cells (MSC) have emerged as an alternative to neuroprogenitors in cell therapy protocols for Parkinson's disease (PD). In this pre-clinical study, we provide evidences supporting the use of human umbilical cord-derived MSC (hUC-MSC) to inhibit chronic neurodegeneration, based on neuroprotective effects in an experimental model of PD. Stereology of tyrosine hydroxylase (TH) positive neurons and behavioral tests indicated that intrastriatal injection of human hUC-MSC in rats previously exposed to the neurotoxin 1-metyl, 4-phenyl, 1, 2, 3, 6-tetrahydropyridine (MPTP) preserved the amount of dopaminergic neurons in the substantia nigra (SN) and prevented onset of motor deficits 30 days post-MPTP exposure. Immunohistochemistry with fluorescent antibodies against human nucleous and against TH revealed rare engrafted cells with dopaminergic phenotype, suggesting poor neuronal differentiation in vivo. However, TUNEL assays indicated lower amount of apoptotic neurons in rats treated with hUC-MSC compared with control animals (sham). Moreover, after six months of follow up, quantitative analysis by stereology revealed that treatment with hUC-MSC significantly inhibited chronic dopaminergic neurodegeneration in animals exposed to MPTP. Surprisingly, the amount of TH+ neurons in the SN of hUC-MSC-treated animals was also significantly higher than the amount detected in healthy control animals not exposed to MPTP, suggesting protection from neurodegeneration due to ageing. Altogether, these results suggest that a single intrastriatal injection of hUC-MSC at early stages of the MPTP model induces potent neuroprotective adjustments in the brain and long-term inhibition of dopaminergic neurodegeneration. These findings provide a rationale for further clinical trials with hUC-MSC aiming at delaying disease progression and intensification of motor deficits.

F-3092

EXPANSION AND DIFFERENTIATION OF WHARTON'S JELLY MESENCHYMAL STEM CELLS INTO RENAL TISSUE

Maha A. El Mansy[1], Amira R. Harb[1], Tahany A. Alkerdany[2], Nariman M. Zahran[1], Hanan M. Mahmoud[2]

[1]Clinical Pathology, Theodor Bilharz Research Institute, Cairo, Egypt, [2]Clinical Pathology, Ain Shams University, Cairo, Egypt

Stem cells are non specialized cells that have the ability of self renewal and give rise to specialized mature cell types or tissues like bone, cartilage, adipose tissue and muscles. Stem cell remains non specialized until they get signals like growth factors to be converted into specialized cells. They can be isolated from different sources of human tissues such as bone marrow, peripheral blood, placenta, umbilical cords and embryonic stem cells in order to be used therapeutically to regenerate or replace any tissue or organ.

The aim of the present study is to use Wharton's Jelly of the umbilical cord as an easy accessible source for isolation of mesenchymal stem cells (MSCs), in addition to in vitro expansion and differentiation of these cells into renal tissue.

This study was conducted on 6 umbilical cords obtained from 6 caesarian section (CS) deliveries.The MSCs were isolated, and immunophenotyping of these cells were done by immunocytochemistry and flowcytometric analysis for detection of CD29 expression.These cells were expanded and directed toward differentiation in-vitro into renal progenitor stem cells by addition of different cocktails of nephrogenic growth factors such as Retinoic acid, Activin-A and BMP-7. The newly formed renal progenitor stem cells were detected by positive expression of CD24 & CD133 by immunocytochemistry and flowcytometric analysis, the best growth factors cocktail were determined.

There is significant positive expression of CD29 on the cells isolated from the umbilical cord, which is a MSCs surface marker, also there is positive expression of CD24 & CD133 after induction of differentiation by two of the used nephrogenic growth factors coktails, which are renal progenitor stem cell surface marker.

There is a possibility to isolate MSCs from Wharton's Jelly of the umbilical cord, which is an easy accessible source for MSCs. These cells can be differentiated into renal progenitor stem cells by using growth factors cocktail containing a combination of (Retinoic acid + Activin-A) at the begining then BMP-7 is added later. These primitive renal cells may be further utilized by infusion or injection into patients with acute or chronic renal diseases for possible improvement of their condition

F-3093

SERPINE2 PROMOTES OSTEOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS VIA PI3K/AKT AND ERK PATHWAY

Hye-Jeong Park[1], Joo-Hyun Kim[1], Eun-Hwang Lee[1], Tae-Geon Kwon[2], Je-Yoel Cho[1]
[1]Coll of Veterinary Med, Seoul Natl Univ, Seoul, Korea, Republic of, [2]School of dentistry, Kyungpook Natl Univ, Seoul, Korea, Republic of

**Human
bone marrow stem cells (hBMSCs) are the useful sources for tissue engineering. In this study, we examined the secretome of hMSC from mandibular bone (Mn-hMSCs) during early osteogenesis by geLC-MS/MS. We then examined the expression patterns of selected candidates in mRNA levels. Secretion levels of SERPINE1 and SERPINE2 were significantly up- and down- regulated at 3 days of osteogenic induction, respectively. Among these secreted proteins, SERPINE2 mRNA levels were decreased and then elevated 7 days after osteogenic differentiation induction. This pattern of SERPINE2 expression was also confirmed in secreted protein levels during osteogenic diffentiation. SERPINE2 gene expression was induced by TGFβI and BMP2 gene was increased by SERPINE2 suggesting that SERPINE2 plays a role as switch of proliferation to differentiation. To examine the role of SERPINE2 in osteogenic differentiation, we treated the Mn-hMSCs with human recombinant SERPINE2 protein (rhSERPINE2) with osteogenic medium. rhSERPINE2 significantly enhanced mineralization of Mn-hBMSCs and increased osteogenic specific genes that can be induced by BMP2. rhSERPINE2 significantly increased phsopho-Akt and phospho-Erk1/2
level. In addition, rhSERPINE2-induced Akt and Erk1/2 activity and ALP activity were blocked by PI3K and Erk inhibitors, respectively. In conclusion, SERPINE2 is supposed to be involved in osteogenic differentiation via PI3-Akt and Erk pathways and play a role as switch of proliferation to differentiation of mesenchymal stem cells.**

F-3094

S-NITROSYLATION REGULATES THE BALANCE OF ADIPOGENESIS AND OSTEOGENESIS IN MESENCHYMAL STEM CELLS

Yenong Cao, Samirah A. Gomes, Erika B. Rangel, Ellena C. Paulino, Wayne Balkan, Joshua M. Hare
Interdisciplinary Stem Cell Institute, Univ of Miami, Miller School of Medicine, Miami, FL, United States

Introduction: Osteoblasts and adipocytes arise from the same progenitor, multipotent bone marrow-derived mesenchymal stem cells (BMMSCs). Disruption of the differentiation balance between adipocyte and osteoblast lineages is involved in numerous bone and metabolic diseases. Increased bone marrow adipogenesis accompanied by decreased bone mineral density occurs with aging and in diabetic patients following usage of thiazolidinedione, a peroxisome proliferator- activated receptor γ (PPARγ) agonist. Nitric oxide (NO) is an important regulator of skeletal homeostasis. We hypothesized that S-nitrosylation mediated-NO signaling controls the balance between adipogenic and osteogenic differentiation of BMMSCs. **Methods:** Body weight and bone mineral density were measured in wild type mice (WT) and mice deficient in S-nitrosoglutathione reductase ($GSNOR^{-/-}$), a denitrosylase that governs levels of protein S-nitrosylation. BMMSCs were isolated from WT and $GSNOR^{-/-}$ mice. Cells were cultured in either adipogenic or osteogenic differentiation media followed by functional and gene expression assays. S-nitrosylation was tested by SNO-RAC assay. **Results:** $GSNOR^{-/-}$ mice had decreased body weight (22.53±0.45g in WT vs. 18.62±0.51g in $GSNOR^{-/-}$ mice, $P<0.001$) and bone mineral density (46.18±0.66 mg/cm^2 in WT vs. 42.08± 0.61 mg/cm^2 in $GSNOR^{-/-}$ mice, $P<0.05$). BMMSCs derived from $GSNOR^{-/-}$ mice had reduced fat droplet formation and expression of the adipogenic markers *PPARγ* (1329±415.3-fold increase in WT vs. 158±65.61-fold in $GSNOR^{-/-}$, $P<0.05$) and *FABP4* (11.06±3.29-fold in WT vs. 4.06±0.62-fold in $GSNOR^{-/-}$, $P<0.05$). Conversely, $GSNOR^{-/-}$ BMMSCs exhibited

enhanced osteogenic differentiation as indicated by greater calcium deposition and higher expression of the osteogenic marker *Osteopontin* (1.48±0.17-fold in WT vs. 16.18±5.26-fold in GSNOR$^{-/-}$, P<0.05). Interestingly, GSNOR$^{-/-}$ cells had higher baseline expression of *Osteopontin, Osteocalcin* and *Runx2*. Treatment of GSNOR$^{-/-}$ BMMSCs with L-NAME, a nitric oxide synthase (NOS) inhibitor, decreased osteogenic differentiation without affecting adipogenic differentiation. Baseline level of S-nitrosylated PPARγ, a master regulator of adipogenesis and osteogenesis, was increased in GSNOR$^{-/-}$ BMMSCs. **Conclusion:** Denitrosylation via GSNOR regulates the balance between adipogenic and osteogenic differentiation of BMMSCs by affecting the transcriptional and post-translational modification of PPARγ. **Implication:** GSNOR-mediated S-nitrosylation is a pivotal, yet previously unrecognized, regulator of skeletal homeostasis, which may facilitate the development of new therapeutic approaches against bone and metabolic diseases.

F-3095

POSSIBLE IMPACT OF MSC IN WOUND HEALING DISRUPTION AFTER SULFUR MUSTARD EXPOSURE

Annette Schmidt, Horst Thiermann, Dirk Steinritz
Bundeswehr Institute for Pharmacology and Toxicology, Munich, Germany

Introduction:
Sulfur mustard, also known as mustard gas was first used in World War I as a chemical warfare agent. Still up today over the whole world tons of mustard gas do exist and they are still an existing risk. Most important mode of action is the induction of large skin wounds. Sulfur mustard induced skin wounds demonstrate a chronic wound healing disruption, which persist over long time. Mesenchymal stem cells (MSC) are known to play an important role in wound healing. On the other hand it is also known that patients with chronic wound healing diseases do have a disrupted functionality of mesenchymal stem cells. Based on these facts and the known relationship between wound healing disruption and MSC we wanted to ask the question which impact sulfur mustard do have on MSC.
Material&Methods:
Mesenchymal stem cells (MSC) were isolated from femoral head of healthy donors. They were cultured for not more then three passages. MSC were exposed with and without sulfur mustard in different concentrations. After exposure we analyzed induction of apoptosis, cell survival, cell aging, migration ability and capacity of tissue specific differentiation.
Results:
MSC demonstrated an unexpected high tolerance against toxic concentrations of sulfur mustard, which is not known from any other kind of cells. On the other hand gas exposure showed a negative effect onto the migration ability. An increased induction of apoptosis was not observed, whereas the cells demonstrated an accelerated senescence. Also an effect on differentiation capacity was not observed.
Discussion:
The effect of sulfur mustard onto MSC might play an important role in the long-term persistent adverse effects. Our current results will help to understand the relationship and also give an assistance in the future perspective for therapeutic use of MSC within patients with sulfur mustard induced chronic wound healing disruption.

F-3096

OVEREXPRESSION OF CYCLIN D2 PROMOTES CELL PROLIFERATION OF HUMAN MESENCHYMAL STEM CELLS

Ken Kono, Rumi Sawada, Atsuko Matsuoka
Division of Medical Devices, National Institute of Health Sciences, Tokyo, Japan

Human mesenchymal stem cells (hMSC) can differentiate into various tissues such as bone, cartilage, muscle, ligament, tendon, and adipose. Due to this ability, hMSC are expected for cell-based therapies and tissue engineering. However, to obtain the enough number of cells for clinical protocols, we have to extract hMSC from our bodies and culture them in vitro. Although hMSC are thought to be stable even in ex vivo, there is no denying the possibility that undesirable transformation occurs during the ex vivo expansion. In this study, we performed the whole gen-

ome expression analysis of hMSC by using cell lines from Ewing sarcoma, which are thought to be derived from hMSC, as a positive control and found that Cyclin D2 is overexpressed in the Ewing sarcoma cells. Cyclin D2 was reported be overexpressed in various tumors and considered to be involved in cell tumorigenesis. Thus, we transduced Cyclin D2 into hMSC. Overexpression of Cyclin D2 altered cell morphology and promoted cell proliferation, but it did not lead to unlimited proliferation. Furthermore, real-time PCR analysis showed that the induction rate of p16 gene, which is involved in the cellular senescence, was faster than that of control hMSC. These results suggested that Cyclin D2 was important for the accelerated proliferation but not for the malignant transformation of hMSC. Now we are performing microarray analysis of Cyclin D2-transduced hMSC to search what pathways were affected by the overexpression.

F-3097

DECODING THE SECRET LANGUAGE OF SURFACE TOPOGRAPHY INDUCED STEM CELL DIFFERENTIATION

Frits Hulshof[1], Marc Hulsman[2], Shantanu Singh[3], Bernke Papenburg[1], Marloes Levers[1], Mark-Anthony Bray[4], Clemens van Blitterswijk[1], Marcel Reinders[2], Anne Carpenter[4], Dimitris Stamatialis[1], **Jan de Boer**[1]
[1]MIRA Institute for Biomedical Technology and Technical Medicine, Univ of Twente, Enschede, Netherlands, [2]Technical University of Delft, Delft, Netherlands, [3]Broad Institute of MIT and Harvard, Cambrdige, MA, United States, [4]Broad Institute of MIT and Harvard, Cambridge, MA, United States

We all know that cells can respond to diffusible molecules but it is less well known that they are also able to respond to a secret language consisting of geometric patterns of surface topography. To unravel this secret Braille language, we have designed and engineered a library of surface topographies, reproduced onto polymeric surfaces, known as the TopoChip. Using high-content imaging, we are now analyzing the response of human mesenchymal stromal cells to thousands of different surface patterns. We have found surfaces which induce expression of the early osteoblast marker alkaline phosphatase (ALP) to levels similar to that induced by classical osteoblast inducers such as dexamethasone. In addition to ALP staining, we have also stained the actin cytoskeleton and the nucleus, and using CellProfiler software, we have extracted nearly 300 morphological features for each cell on the TopoChip. Using machine learning algorithms, we are now able to predict ALP expression based on cell morphology alone, and further experiments are in progress to investigate a possible correlation between the actin cytoskeletal organization and ALP expression. In conclusion, using a high-throughput screening approach, we can now start to unravel the secret language of surface topographies.

F-3098

ENHANCED MESENCHYMAL STEM CELL MIGRATION AFTER GSK-3BETA INHIBITION DURING EX VIVO EXPANSION

Young Seo Kim[1], Seung-Hyun Kim[1], Seong-Ho Koh[1], Min Young Noh[1], Won Jun Choi[1], Ki-Wook Oh[1], Seong-Il Oh[1], Kyung Suk Kim[2], In Young Chang[2]
[1]Neurology, College of Medicine, Hanyang University, Seoul, Korea, Republic of, [2]Corestem Inc, Seoul, Korea, Republic of

Mesenchymal stromal cells (MSCs) are stromal cells that are found in a wide range of adult post-natal organs and tissues including bone marrow, blood, and adipose tissues. Since, these cells can be obtained from various tissues, can be expanded easily *ex vivo,* and exert their beneficial effects not only by cell replacement, but also by neuroprotection, trophic support, and angiogenesis, they are considered promising therapeutic stem cell candidates with widely performed clinical trials. However, the majority of MSCs transplanted by various routes fail to reach the site of injury, and they have demonstrated only minimal therapeutic benefit in clinical trials. In this study, we assessed whether inhibition of glycogen synthase kinase-3β (GSK-3β) increases the migration capacity of MSCs during *ex vivo* expansion. Human bone marrow MSCs (hBM-MSCs) were cultured with various GSK-3β inhibitors (LiCl, SB-415286, and AR-A014418). A QCM chemotaxis assay (8 μm pore size, 96-well migration assay) revealed that treatment of hBM-MSCs with various GSK-3β inhibitors consistently increased their migration capacity. Among the GSK-3β in-

hibitors, 10 mM LiCl and 0.25 μM SB-415286 resulted in the highest motility. Additional migration assays using chemoattractant (SDF-1α at 30mg/ml, the most effective dose in our experiments) showed increased migration of the hBM-MSCs compared with those without. However, the beneficial effect of GSK-3β inhibitor treatment was reduced in SDF-1α-induced migration and SB-415286 was the only inhibitor that significantly increased cell migration. Western blot analysis revealed increased levels of migration-related signaling proteins such as phospho-GSK-3β, β-catenin, phospho-c-Raf, phospho-extracellular signal-regulated kinase (ERK), phospho-β-PAK-interacting exchange factor (PIX), and CXC chemokine receptor 4 (CXCR4). In addition, real-time polymerase chain reaction demonstrated increased expression of matrix metalloproteinase-2 (MMP-2), membrane-type MMP-1 (MT1-MMP) and β-PIX. In the reverse approach, treatment with β-PIX shRNA or CXCR4 inhibitor (AMD 3100) reduced hBM-MSC migration. These findings suggest that inhibition of GSK-3β during *ex vivo* expansion of hBM-MSCs may enhance their migration capacity by increasing expression of β-catenin, phospho-c-Raf, phospho-ERK, and β-PIX, and the subsequent up-regulation of CXCR4. Enhancing the migration capacity of hBM-MSCs by treating these cells with GSK-3β inhibitors may increase their therapeutic potential.

F-3101

NANOPILLAR ARRAYS FOR MESENCHYMAL STEM CELL DIFFERENTIATION

Julie A. Morabito, Daniel Ratchford, Junghoon Yeom, Pehr E. Pehrsson, Bradley Ringeisen, Paul E. Sheehan, Shawn P. Mulvaney
Chemistry, Naval Research Laboratory, Washington, DC, United States

Substrate interactions can direct in vitro stem cell differentiation towards desired lineages. Recently, silicon nanopillars have been explored as a versatile cell culture platform for investigating and manipulating intracellular and cell-material processes. It remains unclear, however, how mesenchymal stem cells (MSC) differentiate on nanopillar arrays as compared to planar surfaces. Consequently, we sought to explore the effect of local topography on differentiation. Instead of conventional protein or extracellular matrix (ECM) coatings, both planar surfaces and nanopillar arrays were coated with alumina using atomic layer deposition which was found to enhance cell viability compared to bare silicon. The MSCs were cultured on these surfaces in adipogenic, chondrogenic, osteogenic, and control media, and their morphology characterized after one and two weeks.
Morphological analysis revealed that MSC differentiation responded to the local topography. After one week, MSCs in control media on the nanopillars were not morphologically different from MSCs on flat alumina films. After two weeks, MSC in control media on the nanopillars exhibited similar morphology but a lesser degree of local cell-cell alignment than MSC in control media on flat alumina surfaces.
At one week, MSC in adipogenic media on the nanopillars were more elongated than the round MSCs on the flat substrate. At two weeks, this trend persisted demonstrating a sustained response to the nanopillars.
At one week, MSC in chondrogenic media on nanopillars were more elongated, triangular, and exhibited process-like features when compared to MSC on flat surface, suggesting enhanced chondrogenesis. By two weeks, MSC in chondrogenic media on nanopillars formed several island-like aggregations. In this culture medium, MSCs on both substrates exhibited classic elongated and triangular chondrocyte morphology. This result is consistent with the three-dimensional pellet culture morphology of chondrocytes, and suggests a role for nanopillars in promoting chondrogenesis.
At one week, MSC in osteogenic media on nanopillars exhibited a stellate morphology with rougher borders when compared to MSCs in osteogenic media on flat films, suggesting enhanced osteogenesis on pillars. However, by two weeks, all MSCs in the osteogenic media on both surfaces were unviable, indicating that alumina does not support cell health across all lineages, and further, that substrate conditions should be carefully tailored for each desired lineage.
Taken together, these results support the potential of nanopillars to enhance MSC differentiation along selected cell lineages without the need for protein or ECM surface coatings. Moreover, the established control over nanoarray pitch, length, and diameter, as well as biological and chemical coatings, offers broad avenues for developing materials to control ex vivo MSC differentiation and thus to bolster tissue engineering for regenerative medicine.

F-3102

LNGFR+THY1+CD106+ CELLS REVEAL FUNCTIONALLY DISTINCT SUBPOPULATIONS IN MESENCHYMAL STEM CELLS

Yo Mabuchi[1], Satoru Morikawa[2], Seiko Harada[2], Kunimichi Niibe[2], Sadafumi Suzuki[2], Francois Renault-Mihara[2], Diarmaid D. Houlihan[3], Chihiro Akazawa[1], Hideyuki Okano[2], Yumi Matsuzaki[2]

[1]Tokyo Medical and Dental University, Tokyo, Japan, [2]Keio University School of Medicine, Tokyo, Japan, [3]University of Birmingham, Birmingham, Japan

Mesenchymal stem cells (MSCs) are self-renewing cells with the multipotent ability and considered as potential candidates for a variety of clinical applications. Traditional human MSC isolation methods require prolonged in vitro culture on plastic plates, which reduces their differentiation potential and proliferative ability. Furthermore, so called human MSCs isolated by this traditional method are very heterogeneous and could alter the genuine MSC phenotype, making it difficult to identify specific surface markers that can be used for the aforementioned objectives of the intrinsic characteristics. Thus, it is obvious that the absence of the prospective clonal isolation method of human MSCs have hindered the appropriate development of stem cell biology of human MSCs.
Here we show that the combination of LNGFR+, Thy-1+ and CD106high+ for the prospective isolation of a highly potent population of MSCs from human bone marrow. By using the enriched primary MSCs, single cell assays demonstrated functional hierarchy within clonogenic cells (CFU-Fs). Three distinct subpopulations were identified: rapidly-, moderately- and slowly-proliferating clones (RPCs, MPCs and SPCs, respectively). We identified that RPCs were the most immature stem-like characteristics. Of note MPCs and SPCs showed low differentiation potential, tended to acquire cellular senescence via p16INK4a and exhibited frequent genomic errors. RPCs isolated clonally or based on their unique expression of CD106 will help to improve from cultured MSC or from fresh bone marrow. Furthermore, RPCs showed distinct characters of cellular motility, which required CD106-CD49d interaction.
Collectively, the LNGFR+Thy-1+CD106high+ MSCs established in the present study will be a valuable scientific tool for important milestones in the evolution of the basic and clinical MSC research field.

F-3103

GLOBAL TRANSLATIONAL REPRESSION IS A CRUCIAL REGULATOR OF ADIPOGENIC DIFFERENTIATION OF MESENCHYMAL STROMAL CELLS.

Vivek M. Tanavde[1], Betty Tan[1], Michelle Kwan[1], Prabha Sampath[2]

[1]Bioinformatics Institute, Singapore, Singapore, [2]Institute of Medical Biology, Singapore, Singapore

Understanding differentiation of Mesenchymal Stromal Cells (MSC) is important in regenerative medicine since these cells have wide therapeutic potential. Micro RNAs (miRNAs) regulate self renewal and differentiation of a number of stem cells (Bao 2013, Yu Z 2012). Since miRNAs act post transcriptionally & translational regulation is an important regulator of stem cell differentiation (Sampath P 2008), we investigated the role of translational regulation in trilineage differentiation of Mesenchymal Stromal Cells. Using polysome fractionation, we measured the proportion of RNA being translated in MSC differentiating into bone, cartilage & fat. We observed global suppression of translation during adipogenic differentiation of MSC. Such global suppression of translation is usually associated with a stress response (Paschen W 2007) and accompanied by a reduction in cell division. However in our study such global suppression of translation was not accompanied by inhibition of cell division, since the cell numbers increase during adipogenic differentiation of MSC.
Further we studied the mechanism of translational suppression by measuring the total protein expression and phosphorylation status of proteins involved in the protein translational machinery. We observed a reduction in the phosphorylated form of eIF4E and 4E-BP1 proteins. However there was no change in the phosphorylation status of eIF2a. Thus our results indicate that this global suppression of translation is mediated through the mTOR pathway and not through the alternate eIF2a machinery.
Suppression of translation as a stress response is mediated through mTOR as well as eIF2a (Hofmann S 2012). Since in our cultures the number of cells increases in adipogenic induction medium & the translation suppression is spe-

cifically mediated through mTOR signaling, this is unlikely to be a stress response. This translational suppression is not associated with chondrogenic and osteogenic differentiation. Therefore global suppression of translation may be a specific regulatory mechanism required for adipogenesis. This regulatory mechanism could be exploited for adipogenic differentiation of stem cells. It will be interesting to see if this mechanism regulates lipid biosynthesis as well.

F-3104

TRANSCRIPTIONAL NETWORKS INVOLVED IN DIFFERENTIATION OF RENAL PROGENITOR CELLS TO THE JUXTAGLOMERULAR CELL FATE

Maria Mirotsou, Jose Gomez, Karthikeyan Ardhanareeswaran, Jennifer Chien, Tilanthi Jayawardena, Conrad Hodgkinson
Duke Univ, Durham, NC, United States

The renin-angiotensin-aldosterone system regulates blood pressure and salt-volume homeostasis. Renin, the first and rate-limiting step in the system, is synthesized and released by highly specialized cells in the juxtaglomerular area of the kidney named juxtaglomerular (JG) cells. Despite their physiological importance, the molecular processes regulating the development of JG cells are not fully understood. Here, we present evidence that after stimulation with cAMP or LXRa adult renal MSC-like cells have the capacity to differentiate into renin producing cells with JG cell characteristics. Our objective was to employ this in vitro differentiation system to investigate the transcriptional networks involved in the commitment of progenitor cells to the JG lineage. For this, adult renal CD44+ MSCs were isolated from C57BL/6 Ren1c YFP mice. MSCs were differentiated into YFP+ renin expressing cells by treatment with LXRa ligand (T0901317) or cAMP. The Affymetrix Mouse 430 2.0 array was used for profiling. Data analysis was focused on transcriptional and epigenetic regulators and performed by using Dchip, David or Toppgene. Differentiated YFP+ cells from either cAMP or LXRa treatment expressed high levels of renin and renin regulators such as Hoxd8 and C/ebp. Untreated MSCs showed expression of metanephric markers such as Foxd1, Sox11 and Eya. Treatment with cAMP resulted in differential expression of 108 transcripts in YFP+ cells as compared to control MSC. The majority of these genes (82) were down-regulated and involved in chromatin acetylation (23 out of 68, P<2E-5) or embryonic and hematopoietic development (36 out of 81 respectively, P<2E-5). Mesenchymal markers (i.e. Sox11, FC= -3) and repressors of differentiation (i.e. Id2, FC= -2) were also decreased significantly. Similarly, MSC activation by LXRα resulted in differential expression of 98 transcripts, 67 of which were decreased compared to baseline. As with cAMP, the majority of genes were involved in differentiation and chromatin remodeling (i.e Tbx3, Klfs and Mll); still, the overlap of genes between the two treatments was minimal (24 genes) suggesting that cAMP and LXRa might act complementary to each other. Our data indicate that cAMP and LXRa, rather than directly define JG cell fate, modulate the epigenetic status of MSCs and prevent differentiation to other lineages. Systems biology approaches are under way to evaluate the importance of the transcriptional networks identified. We anticipate that these results will provide novel insights into the mechanisms regulating JG cell fate.

F-3105

OSTEOGENIC DIFFERENTIATION OF STEM CELLS PROMOTED BY THE ASSEMBLIES OF PEPTIDE-DISPLAYED BACTERIOPHAGE

Chuanbin Mao
Chemistry & Biochemistry, University of Oklahoma, Norman, OK, United States

Filamentous phage is a biological nanofiber. It is made of proteins and DNA. There are about ~3000 copies of major coat encapsulating the DNA core. There are some minor coat proteins capping the tips of the filamentous phage. Because DNA inside the phage encodes both the minor coat proteins (at the tip) and major coat protein (on the side wall) of the phage, foreign peptides can be displayed site-specifically on the surface of the filamentous phage by genetic means. We displayed peptides derived from bone proteins including fibronectin and bone morphometric protein 2 on the surface of the filamentous phage. The phage nanofibers were induced to self-assemble into a

nanostructured film where phage nanofibers were parallel to each other. We then studied the proliferation and osteoblastic differentiation of mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs) on the surface of such film. We found that the filamentous phage constituting the film could control the morphology of MSCs and iPSCs. More importantly, the unique architecture of the phage assemblies along with the peptides displayed on the constituent phage could promote the proliferation and osteoblastic differentiation of MSCs and iPSCs. In addition, when some peptides derived from bone proteins were displayed on phage, the phage assemblies could induce the osteogenic differentiation of stem cells without any osteogenic supplements.

F-3106

HUMAN PLACENTA-DERIVED MULTIPOTENT MESENCHYMAL STEM CELLS EXHIBIT UNIQUE FEATURES THAT MAKE THEM IDEAL CANDIDATES FOR CANCER AND WOUND HEALING THERAPIES.

Elvira Herrero de Laorden[1], Francisco Moreno Garzón[1], **Pilar Martin-Duque**[1], Maria del Carmen Turpin[1], Miguel Quintanilla[2], Ester Martín[2], Maite Iglesias[1]

[1]Biotechnology, Francisco de Vitoria´s University, Pozuelo de Alarcón. Madrid, Spain, [2]Instituto de Investigaciones Biomédicas "Alberto Sols", UAM-CSIC, Madrid, Spain

Placenta tissue is studied as an important reservoir of stem cells with great potential in regenerative medicine and cell therapy because of its unique features: phenotypic plasticity, immunomodulatory properties and ease of isolation procedure. This application involves: removal of cells from a donor, cell expansion in culture, administration to the recipient and migratory capacity of cells toward injury in vivo. In vitro cell culture is a tricky step since cells must overcome a "traumatic" situation preserving all its characteristics and properties. This work has focused in determining whether human placenta messenchymal stem cells (phMSCs) maintain their specific phenotypic and genotypic profile and multipotent properties when expanded in culture. Moreover, we studied phMSCs migratory potential both in vitro and in vivo. Our group characterized the expanded phMSCs further culture amplification from a genotypic, phenotypic and functional standpoint. We monitored the proliferative capacity of phMSCs at 10 different passages and in two different cell culture conditions, low density (subconfluence) and high density (confluence); a marked difference in the proliferation ability was observed. An analysis of the genes related to self-renewal and pluripotency Sox-2, Oct-4 and Nanog was performed by qRT-PCR without significant variation in their expression. A similar expression pattern of mesenchymal markers such as CD90, CD105 and CD44 was observed by flow cytometry analysis. We also analyzed the cellular potential to differentiate into adipogenic and osteogenic lineages, together with the evaluation of the hPMSC migratory capacity in vitro and in vivo. Assays including wound healing and trans-well 8 µm pore size assays, in vitro, and tumor and skin wound in animal models, in vivo, were conducted. In our hands significant migratory capacity was observed. Cells were able to cover the injury or to migrate through the 8 µ insert, in vitro. Additionally, cells reached the injury in the animals: in vivo GFP signal tracking and NMR analysis. No big differences in migratory potential were observed when cells from different passes were compared. Our data revealed that phMSCs show a remarkable stability at phenotypic, genotypic and functional level when expanded in culture. These results together demonstrated migratory ability in vivo, and point them as interesting candidates in raising clinical applications

F-3107

PARACRINE EFFECTS OF SYSTEMIC INJECTED HUMAN PLACENTA DERIVED MESENCHYMAL STEM CELLS (HPMSCS) ON RETINAL ANGIOGENESIS

Jisook Moon, Kyung-Sul Kim
CHA Univ, Seoul, Korea, Republic of

Abnormal angiogenesis is a primary cause of many eye diseases, including diabetic retinopathy, age-related macular degeneration, and retinopathy of prematurity. Mesenchymal stem cells (MSC) are currently being investigated as a treatment for retinal diseases based on their neuroprotective and angiogenic

properties. In this study, we evaluated the role of systemically-injected human placenta amniotic membrane-derived MSCs (AMSCs) on pathologic neovascularization of proliferative retinopathy. We confirmed that AMSCs secrete elevated levels of TGF-β, which suppresses the proliferation of endothelial cells under pathological conditions *in vitro*. Moreover, in a mouse model of oxygen-induced retinopathy, intraperitoneally-injected AMSCs migrated into the retina and suppressed excessive neovascularization of the vasculature via expression of TGF-β. These findings provide new insight into the therapeutic function of AMSCs for the treatment of retinal neovascular diseases.

Muscle Cells

F-3111

PROSPECTIVE ISOLATION OF HUMAN SKELETAL MUSCLE PRECURSORS CELLS.

Alessandra Castiglioni[1], Simone Hettmer[2], Tata Nageswara Rao[3], Indranil Sinha[4], Matthew D. Lynes[3], Yu-Hua Tseng[3], Amy J. Wagers[5]

[1]Universita Vita-Salute San Raffaele, Milan, Italy and Joslin Diabetes Center, Boston, MA, United States, [2]Pediatric Oncology, Dana Farber Cancer Institute and Division of Pediatric Hematology/Oncology, Children's Hospital and Joslin Diabetes Center, Boston, MA, United States, [3]Joslin Diabetes Center, Boston, MA, United States, [4]Division of Plastic Surgery, Brigham and Women's Hospital, Boston, MA, United States, [5]Howard Hughes Medical Institute, Department of Stem Cell and Regenerative Biology, Harvard University, Harvard Stem Cell Institute, Joslin Diabetes Center, Boston, MA, United States

Satellite cells are responsible for the maintenance, growth and repair of skeletal muscle. They represent a specialized subset of mononuclear cells within the heterogeneous pool of myofiber-associated (MFA) cells. Satellite cells are located under the basal lamina of muscle fibers and express the transcription factor paired box protein 9 (PAX7). In mice, combinatorial staining of multiple cell surface markers followed by fluorescence activated cell sorting (FACS) allows for isolation of highly enriched, PAX7-expressing myogenic cells, called skeletal muscle precursors (SMPs), from the MFA cell pool. Here, we sought to apply a similar approach to identify combinations of cell surface markers that can discriminate functionally distinct cell lineages within the human myofiber-associated (hMFA) cell compartment, with the final goal of isolating human myogenic precursor cells. Our study of fetal skeletal muscle reveals marked phenotypic and functional heterogeneity within the hMFA pool. We identify two distinct populations of cells: (1) $CD45^-Mac1^-GlyA^-CD31^-CD34^-CD56^{int}ITGA7^{hi}$ hMFA cells represent human skeletal muscle precursor cells (hSMPs). They are highly enriched for PAX7-expressing cells (78± 5%), show extremely efficient myogenic differentiation capacity in vitro and lack adipogenic differentiation potential. (2) $CD45^-CD11b^-GlyA^-CD31^-CD34^+$ hMFA cells ($CD34^+$ cells) do not express PAX7, lack myogenic activity and exhibit adipogenic activity in vitro. Both fetal hSMPs and fetal CD34+ cells show osteogenic activity in vitro. Gene expression profiling confirms that the transcriptional signatures of hSMP and $CD34^+$ cells are profoundly different, with increased expression of muscle lineage genes in hSMPs and adipogenic genes in CD34+ cells. Furthermore, within the pool of hMFA cells isolatable from adult skeletal muscle, $CD45^-Mac1^-GlyA^-CD31^-CD34^-CD56^{int}ITGA7^{hi}$ cells are enriched for PAX7-expressing cells (89± 7% PAX7 positive) and exhibit myogenic and osteogenic activity in vitro. We conclude that FACS sorting of $CD45^-Mac1^-GlyA^-CD31^-CD34^-CD56^{int}ITGA7^{hi}$ cells from fetal or adult skeletal muscle allows prospective isolation of a human skeletal muscle precursor cell population. Protocols to isolate fresh, highly purified human myogenic precursors were not available up until now, and we anticipate that this new technology will provide novel insights into muscle growth and repair in normal homeostasis, aging and disease.

F-3112

USING PHASE CONTRAST MICROSCOPY TO CHARACTERIZE THE MORPHOLOGY AND THE GROWTH KINETICS OF HUMAN MUSCLE PRECURSOR CELLS IN SEVERAL CULTURE MEDIA VIA IMAGE PROCESSING AND MULTIVARIATE TECHNIQUES

Pierre-Marc Juneau, Alain Garnier, Carl Duchesne
Chemical Engineering, Université Laval, Québec, QC, Canada

Phase contrast microscopy (PCM) is a widely used non-intrusive method to monitor cell cultures. Although many existing software are able to analyze fluorescent images, processing a large database of PCM images remains a challenging task because of illumination variations and the low contrast between cellular and background regions. We have developed algorithms to segment and analyze PCM images taken with a long-term, large-field automated live cell imaging platform. An IX81 Olympus® microscope equipped with an automated stage and a built-in incubator was used to monitor human muscle precursor cells (*h*MPCs) grown in either serum supplemented (SSM) or serum-free medium (SFM) over several passages. A total of 1.5 million cell culture images were acquired from seeding to confluence using Metamorph® and analyzed using our custom Matlab® scripts.
A set of 200 ground truth (hand-segmented) images was used to tune a segmentation algorithm for computing the cell-covered surface (degree of confluence) against time and also to calibrate a partial-least square (PLS) model in order to assess the size distribution of the cells within each PCM image from textural features. To assess the growth kinetics of the cells, unbounded and bounded kinetic models were fitted using the cell-covered surface in each well as a function of time. A total of 222 growth curves were analyzed, showing a linear relationship between passage number and the kinetic parameters. For instance, the relationship between the specific growth rate of the exponential model (μ, day-1) and the number of passages (Npassage) is $\mu = 0.958 - 0.0407 * Npassage$. To compute the size distribution of the cells in order to characterize their morphology, a pattern recognition approach was used through the generation of textural features via the undecimated wavelet transform (UWT). The UWT is a multiscale approach in which a quantitative estimate of image details at each scale can be produced (image textural features). The data generated from image processing (cell-covered surface and cells size distribution) was analyzed through multivariate techniques (PLS models) to establish a direct link between the culture medium used (SSM versus SFM, concentration of FBS or concentration of growth factors), the morphology and the growth kinetics of the *h*MPCs throughout passages. A PLS analysis showed that it was possible to distinguish the morphology of cells grown in SSM and SFM in 609,000 pictures with an accuracy of more than 85%. This approach showed that the impact of the culture medium on the morphology and on the growth kinetics of stem cells can be assessed quantitatively on a very large dataset of PCM pictures using advanced image processing and multivariate techniques. The proposed software can therefore be used for online monitoring and quality control of stem cell cultures observed in PCM and live-cell imaging experiments without the use of fluorescent dyes, such as the screening of serum replacement factors.

F-3113

GENERATING A SCALABLE IPSC-BASED MODEL OF NEUROMUSCULAR DISEASE FOR USE IN DRUG DISCOVERY

Ramzey J. Abujarour, Monica Bennett, Tom Lee, Kevin Lai, Mike Fitch, Chrissie Lynch, Thuy Le, David Robbins, Peter Flynn
Fate Therapeutics, Inc., San Diego, CA, United States

The muscular dystrophies are a collection of progressive myopathies with diverse genetic bases and clinical manifestations. Several experimental therapies, mainly focused on the most-prevalent X-linked diseases are being investigated; however, no effective treatment developed specifically for a muscular dystrophy is currently available. One of the challenges of therapeutic discovery and translation for the muscular dystrophies is the lack of effective preclinical models or cellular systems for drug screening. Mature muscle myofibers cannot be expanded in culture systems and the self renewing stem cell population in muscle, satellite stem cells become activated and differentiate when introduced to in vitro culture, preventing the derivation of a scalable, disease-specific cellular system.

iPSC technology has previously been used for generating disease specific cellular models and is an ideal technology to capture the genetic diversity of the muscular dystrophies in expandable culture system. However effective therapeutic screening will require high quality, homogeneously differentiated cultures of myotubes, myoblasts or muscle progenitor cells. We have previously described high-throughput iPSC derivation and feeder-cell-free single cell culture expansion methods. Using these systems coupled to an inducible differentiation system we generated 16 iPSC lines from fibroblasts of four patients with Duchenne and Becker muscular dystrophies. To generate a homogeneous muscle cell culture, we devised an efficient and expedited protocol for the differentiation of iPSCs as a monolayer through the inducible overexpression of the muscle master regulator MYOD. Patient-derived multinucleated myofibers could be obtained at high efficiency from iPSCs within a week following MYOD induction as evident by suppression of pluripotency markers and induction of the musculoskeletal program. Differentiation into myocytes was mediated by MYOD soon after induction and did not require a priming stage. To evaluate the potential of the muscular dystrophy model as a platform for drug discovery, we treated cultures with insulin-like growth factor and Wnt7a and assessed hypertrophic effects. Treatment of the dystrophic iPSC-derived myofibers with either of the recombinant proteins induced significant hypertrophy confirming the potential of our Wnt7a therapeutic in a human model of disease. These results validate our iPSC-based model of muscular dystrophy and establish its utility for studying disease development and for drug discovery.

F-3114

NOTCH SIGNALING ENHANCES SELF-RENEWAL IN EMBRYONAL RHABDOMYOSARCOMA

Myron Ignatius[1], Eleanor Chen[2], Karin McCarthy[2], Brian Belyea[3], Corinne M. Linardic[3], Charles Keller[4], David Langenau[1]

[1]Massachusetts General Hosp/Harvard Stem Cell Institute, Charlestown, MA, United States, [2]Massachusetts General Hosp, Charlestown, MA, United States, [3]Departments of Pediatrics, Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC, United States, [4]Pape' Family Pediatric Research Institute, Department of Pediatrics, Oregon Health & Science University, Portland, OR, United States

Embryonal rhabdomyosarcoma (ERMS) is a devastating pediatric cancer of muscle. Relapse is the major clinical challenge facing patients with ERMS, with 50% of patients succumbing to their disease. Thus, there is a clinical imperative to identify pathways that kill relapse-associated cells and/or induce them to differentiate into non-proliferative cell types. Work from our group has recently identified the tumor-propagating cell (TPC) in zebrafish ERMS that is responsible for driving relapse. The TPC is molecularly similar to a muscle satellite cell that expresses myf5, c-met, and m-cadherin. Building on these observations, we have identified the Notch pathway as a modulator of TPC function in ERMS. Specifically, we have compared zebrafish ERMS that transgenically over-express only kRASG12D with those that co-express activated kRASG12D and Intracellular Notch1 (ICN1). Limiting dilution cell transplantation experiments revealed a 10-fold increase TPCs contained within the bulk of the tumor mass in ICN1- expressing ERMS when compared with those that express only RAS (n=3 ERMS/genotype). This increase in TPC number was associated with an increase in the relative expression of satellite cell associated genes including myf5 and c-met. ERMS was also generated in fluorescent transgenic reporter lines that label muscle cells based on differentiation status. From analysis of these animals, we observed a dramatic 3-fold increase in myf5-GFP+/mylz2-mCherry-negative TPCs - the population of cells previously shown to drive relapse growth in RAS-driven ERMS. Subsequent limiting dilution cell transplantation experiments using sorted GFP+/mCherry-negative also documented a 3-fold increase in self-renewal potential within this gated cell population in ERMS that coexpress kRASG12D and ICN1 (average of 1:50 vs. 1:146, n=2 ERMS/genotype). Unexpectedly, Notch also conferred tumor propagating ability to myf5-GFP+/mylz2-mCherry+ cells, a population of cells that are unable to generate tumor in RAS alone expressing ERMS. Next, we assessed a role for NOTCH in regulating self-renewal and differentiation in human ERMS. We find that transduction of human RD cells with ICN1 resulted in increased sphere-colony formation, a surrogate for self-renewal in vitro. By contrast, shRNA knockdown of NOTCH1 resulted in decreased sphere-colony formation ($p=0.048$) and induced robust terminal differentiation of ERMS RD cells into late-stage, myosin-expressing myoblasts (2.7±1.1% in controls vs. 27.7±2.9% in knockdown cells). Limiting dilution experiments in mice using xenograft transplantation

of human ERMS cells that over-express ICN1 and have a knock-down of NOTCH1 are currently underway. Taken together, our data indicate that Notch signaling is an important modifier of ERMS - acting to regulate both TPC self-renewal and differentiation.

F-3115

RECIPROCAL INTERACTIONS BETWEEN MUSCLE-RESIDENT SATELLITE CELLS AND FIBROADIPOGENIC PRECURSORS IN AGE-DEPENDENT CONTROL OF MYOGENESIS AND ADIPOGENESIS

Rohan R. Manohar[1], Danika Khong[2], Elizabeth Wu[2], Kah-Yong Tan[2], Young Jang[2], Amy Wagers[3]
[1]Islet Cell and Regenerative Cell Biology, Joslin Diabetes Center, Harvard Medical School, Boston, MA, United States, [2]Department of Stem Cell and Regenerative Biology, Harvard University, Harvard Stem Cell Institute, Boston, MA, United States, [3]Joslin Diabetes Center, Harvard Medical School, Harvard Stem Cell Institute, Howard Hughes Medical Institute, Boston, MA, United States

Lifelong maintenance of muscle mass and regenerative potential depends on the continuing presence of a functional pool of muscle satellite cells, which serve as self-renewing stem cells for skeletal myofibers. Loss of satellite cells and defects in satellite cell activity are associated with a wide variety of neuromuscular disorders, including muscular dystrophy, spinal muscular atrophy, and sarcopenia of aging. Regeneration of skeletal muscle also is impaired in settings of obesity and diabetes, where accumulation of intramyofibrillar lipid and ectopic adipogenesis contribute to impaired myogenic and metabolic function.
Recent studies suggest that the balance between pro-myogenic and pro-adipogenic signals in muscle is controlled in part by cellular cross-talk between muscle-resident satellite cells and muscle-localized fibro-adipogenic precursors (FAPs), suggesting that therapeutic strategies aimed at manipulating these interactions may be beneficial for promoting myogenesis and restraining fat deposition. Here, we have investigated soluble molecules that may serve as signaling intermediates in the communication between myogenic and fibro-adipogenic cells in muscle. We show that FAP-conditioned medium significantly enhances satellite cell survival in clonal assays. In addition, we found that IL6, Wnt3A and Wnt5A are not responsible for FAP-mediated stimulation of satellite cell myogenesis, and that FAPs from young (2 month old) and old (24 month old) mice show equivalent activity in stimulating myogenesis. We are currently performing proteomics analysis and evaluating the transcriptional profiles of FAPs to identify additional, novel factors that stimulate myogenesis and evaluating the efficacy of myofiber-mediated inhibition of adipogenesis in young and old mice and in mice maintained on high-fat diets. Together, these experiments will provide new insights and possible therapeutic strategies for muscle disease, and may cross-fertilize similar approaches in other organ systems.
This work was funded in part by grants from the NIH and GlaxoSmithKline.

F-3117

MODELING NEUROMUSCULAR DISEASE USING SKELETAL MUSCLE DERIVED FROM DISEASE SPECIFIC INDUCED PLURIPOTENT STEM CELL AGGREGATE CULTURES (EZ SPHERES)

Jered V. McGivern[1], Jonathan M. Van Dyke[3], Tohru Hosoyama[3], Jennifer L. Strande[2], Masatoshi Suzuki[3], Allison D. Ebert[1]
[1]Medical College of Wisconsin, Milwaukee, WI, United States, [B]Division of Cardiovascular Medicine, [1]Medical College of Wisconsin, Milwaukee, WI, United States, [2]Comparative Biosciences, University of Wisconsin, Madison, WI, United States

Pluripotent stem cells (PSCs) provide a valuable source of tissue for generating skeletal muscle progenitor cells (SMPCs). We have previously shown a novel protocol for derivation of SMPCs from human PSCs using spherical aggregates called EZ spheres. Spherical aggregation was developed by lifting intact human PSC colonies and culturing them in a maintenance medium containing high concentrations of human basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF). Human PSCs-derived EZ spheres express paired box transcription factors Pax3 and

Pax7 after acute plate down and expanded culture leads to expression of markers of terminal muscle specification such as myogenin and myosin heavy chain. The derivation of these myogenic cells requires bFGF for the expansion of the pool of myogenic progenitors, and although EGF does not appear to be sufficient for myogenic proliferation under these conditions, EGF may promote differentiation. We used this technique to generate SMPCs and muscle cells from patient-derived PSCs with neuromuscular diseases including Amyotrophic Lateral Sclerosis, Spinal Muscular Atrophy, and Becker's Muscular Dystrophy. Initial examination has not shown significant differences in developmental derivation in the disease-specific lines compared to wild type cell lines. Further investigation of biochemical pathways involved in energy homeostasis and neuromuscular junction formation may provide clues as to the involvement of the skeletal muscle in disease progression. Importantly, the suspension culture protocol provides a tissue source for in vitro disease modeling of neuromuscular diseases.

F-3118

INHIBITING CHRONIC SKELETAL MUSCLE INFLAMMATION IN AGING RESTORES THE STEM CELL NICHE AND IMPROVES REGENERATION

Juhyun Oh[1], Indranil Sinha[2], Kah Yong Tan[1], Min Liu[3], Elisabeth Barton[3], Steven Shoelson[4], Amy Wagers[1]
[1]Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States, [2]Division of Plastic Surgery, Brigham and Women's Hospital, Boston, MA, United States, [3]School of Dentistry, University of Pennsylvania, Philadelphia, PA, United States, [4]Joslin Diebetes Center, Boston, MA, United States

Adult skeletal muscle has the remarkable ability to regenerate, but this ability becomes increasingly compromised with aging and may ultimately contribute to the onset of sarcopenia. Previous work suggests that a decline in skeletal muscle precursor (SMP) myogenic potential may be secondary to age-related chronic skeletal muscle inflammation, mediated by an increase in nuclear factor kappa B activity (NF-kB). Here, we confirm that NF-kB is up-regulated in aged skeletal muscle and that SMPs isolated from this environment exhibit diminished myogenic potential in comparison to their young counterparts. Furthermore, our transgenic model aged with inhibition of NF-kB activity in skeletal muscle fibers shows improved muscle regeneration after injury and myogenic potential of SMPs. However, isolated NF-kB activation in SMPs did not affect their myogenic potential, indicating a non-cell autonomous effect of NF-kB on SMPs. We also report that pharmacologic inhibition of NF-kB with sodium salicylate decreases inflammatory markers and is able to reverse age-associated deficit in skeletal muscle regeneration. Sodium salicylate may therefore be of therapeutic benefit to elderly patients with reduced regenerative capacity in skeletal muscle.

F-3121

FETAL SKELETAL MUSCLE PROGENITORS HAVE REGENERATIVE CAPACITY AFTER INTRAMUSCULAR ENGRAFTMENT IN DYSTROPHIN DEFICIENT MICE.

Hiroshi Sakai[1], Takahiko Sato[1], Hidetoshi Sakurai[2], Takuya Yamamoto[3], Kazunori Hanaoka[4], Didier Montarras[5], Atsuko Sehara-Fujisawa[1]
[1]Department of Growth Regulation, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan, [A]Department of Clinical Application, [B]Department of Reprogramming Science, [2]Center for iPS cell Research and Application, Kyoto University, Kyoto, Japan, [3]Department of Bioscience, Kitasato University School of Science, Kanagawa, Japan, [4]Molecular Genetics of Development, Institut Pasteur, Paris, France

Muscle satellite cells (SCs) are stem cells that reside in skeletal muscles and contribute to regeneration upon muscle injury. SCs arise from skeletal muscle progenitors expressing transcription factors Pax3 and/or Pax7 during embryogenesis in mice. However, it is unclear whether these fetal progenitors possess regenerative ability when transplanted in adult muscle. Here we address this question by investigating whether fetal skeletal muscle progenitors (FMPs) isolated from Pax3GFP/+ embryos have the capacity to regenerate muscle after engraftment into

Dystrophin-deficient mice, a model of Duchenne muscular dystrophy. The capacity of FMPs to engraft and enter the myogenic program in regenerating muscle was compared with that of SCs derived from adult Pax3GFP/+ mice. Transplanted FMPs contributed to the reconstitution of damaged myofibers in Dystrophin-deficient mice. However, despite FMPs and SCs having similar myogenic ability in culture, the regenerative ability of FMPs was less than that of SCs in vivo. FMPs that had activated MyoD engrafted more efficiently to regenerate myofibers than MyoD-negative FMPs. Transcriptome and surface marker analyses of these cells suggest the importance of myogenic priming for the efficient myogenic engraftment. These results are discussed in the context of cell therapy with the pluripotent stem cells derived myogenic progenitors.

F-3122

STAT3 REGULATION OF SKELETAL MUSCLE STEM CELLS

Tufan Aydogdu, Alessandra Sacco
Muscle Development and Regeneration Program, Sanford-Burnham Medical Research Institute, La Jolla, CA, United States

Skeletal muscle stem cells (MuSC) are responsible for the maintenance, repair, and postnatal regeneration of skeletal muscle. These cells reside in a quiescent state beneath the basal lamina surrounding myofibers and become activated in response to injury or trauma to efficiently repair the damaged tissue. Activation and differentiation of MuSC are biological processes regulated by a family of proteins known as myogenic regulatory factors, such as MyoD. A more clear understanding of the regulatory network underpinning the function of MuSC might help identifying potential therapeutic targets for muscle wasting diseases. Among the molecular pathways implicated in adult muscle regeneration, the IL-6/STAT3 signaling cascade plays a major role in hypertrophy of skeletal muscle fibers. Currently, we are investigating its role in the regulation of MuSC function. Our experiments provide evidence that in response to injury STAT3 is actively phosphorylated in vivo in myofibers and the skeletal muscle microenvironment. In addition, we detected increased pSTAT3 in proliferating MuSC in the early phases of their activation upon muscle injury. Based on these findings, we hypothesize that STAT3 plays a dynamic role in MuSC activation by regulating myogenic regulatory factors. Indeed, loss of function studies using short hairpin RNA in MuSC resulted in downregulation of MyoD gene transcription and protein levels, suggesting that STAT3 is an upstream regulator of MyoD. Upon exposure to IL-6 in vitro, MuSC upregulated MyoD RNA and protein expression and this process was STAT3 dependent. Chromatin Immunoprecipitation (ChIP) analyses revealed that STAT3 directly binds to the MyoD proximal enhancer. We confirmed the STAT3-dependent MyoD upregulation by luciferase reporter assays. Surprisingly, STAT3 loss of function significantly promoted proliferation of MuSC. In order to decipher the role of STAT3 in MuSC-mediated regeneration, we performed transplantation experiments into acutely injured mice. Preliminary results demonstrated that in the absence of STAT3, the ability of MuSC to contribute to tissue regeneration is impaired, indicating that STAT3 is required for myogenesis in vivo. Together, these results demonstrate that STAT3 regulates MyoD in MuSC and might play a major role in integrating the extracellular cues promoting MuSC activation, skeletal muscle repair and homeostasis in vivo.

Cardiac Cells

F-3131

HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES FUNCTIONALLY VALIDATE NOVEL ANTIARRHYTHMIC DRUG THERAPY

Kenta Nakamura[1], Moshe Rav Acha[2], Shiro Baba[3], Robert W. Mills[2], Jordan S. Leyton-Mange[2], Patrick T. Ellinor[2], Bruce R. Conklin[4], Sean M. Wu[5], David J. Milan[2]
[A]Department of Medicine, [B]Cardiovascular Research Center, Division of Cardiology, [1]Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States, [2]Department of Pediatrics, Graduate School of Medicine, Kyoto University, Kyoto, Japan, [3]Gladstone Institute of Cardiovascular Disease, University of California, San Fran-

cisco, San Francisco, CA, United States, [4]Stanford Cardiovascular Institute, Stanford University School of Medicine, Stanford, CA, United States

Background: Induced pluripotent stem (iPS) cells provide an immortal supply of somatic cell types for *in vitro* investigation. Derivation of disease-specific iPS cells is a powerful method to model congenital disorders, providing a translational platform for cell-based drug discovery and preclinical testing. Long QT (LQT) syndrome is a lethal cardiac arrhythmia characterized by prolonged cardiac repolarization responsible for 4,000 US deaths annually. There are no targeted pharmacotherapies for LQT syndrome and investigation is limited by the lack of a human disease model. The novel small molecule compound, 2MMB, ameliorates LQT syndrome in a zebrafish model, but its effect on human LQT syndrome is not known. We sought to characterize the electrophysiologic effects of 2MMB in human iPS-based models of LQT syndrome.
Hypothesis: Disease-specific cardiomyocytes derived from patients with LQT syndrome recapitulate the electrophysiologic disease phenotype and provide a robust translational platform for functional drug testing.
Methods: Fibroblasts from patients with LQT2 (*KCNH2*/A422T) and LQT3 (*SCN5A*/N406K) were retrovirally reprogrammed into iPS cell lines using the genes *Sox2, Oct3/4, Klf4* and *c-Myc* and differentiated into cardiomyocytes (iPS-CMs). Whole cell current and voltage clamp recording techniques were used to assess the effect of 2MMB treatment (10 uM, 10 minutes) on cardiac repolarization.
Results: LQT mutations were confirmed by DNA sequencing of patient fibroblasts, iPS cells, and iPS-CMs. All patient-specific iPS lines successfully differentiated into beating cardiomyocytes. Action Potential Duration at 90% (APD_{90}) for LQT2, LQT3 and control iPS-CMs were 602±107 ms, 719±127 ms and 412±76 ms (n=10, $p<0.05$), respectively. Early After Depolarizations (EADs) were observed for LQT2 and LQT3 iPS-CMs but not in control iPS-CMs. LQT2 iPS-CMs have a significantly decreased rapid inward rectifying potassium current (I_{Kr}) of 0.76±0.34 pA/pF compared to 1.4±0.33 pA/pF in control cells (n=5, $p<0.01$). LQT3 iPS-CMs have a significantly increased mean late sodium current (I_{Na}) of 2.3±0.9% of peak I_{Na} compared to 0.5±0.2% of peak I_{Na} in control cells (n=5, $p<0.05$). These findings are consistent with current heterologous models of LQT2 and LQT3. Treatment with the candidate drug 2MMB caused a non-significant APD_{90} shortening in WT cells of 15.5±2.0% (n=18, $p>0.05$), while shortening LQT2 APD_{90} of 34.9±1.0% (n=12, $p<0.05$), and LQT3 APD_{90} of 24.3±1.7% (n=20, $p<0.05$), respectively. The APD_{90} shortening effect was frequency dependent with the greatest effect at slower beating frequencies. No EADs were observed in LQT2 and LQT3 iPS-CMs after 2MMB treatment.
Conclusions: Disease-specific iPS-CMs recapitulate the electrophysiological characteristics of LQT syndrome types 2 and 3 *in vitro*. The novel small molecule 2MMB shortens repolarization in a reverse rate dependent fashion and suppresses arrhythmogenic EADs. These properties of 2MMB treatment may be protective in genetic and acquired forms of LQT syndrome. Disease-specific iPS-CMs provide a robust and scalable platform for *in vitro* cardiac disease modeling and functional drug validation.

F-3132

TRANSMEMBRANE PROTEIN 88: A WNT REGULATORY PROTEIN THAT SPECIFIES CARDIOVASCULAR PROGENITOR CELL FATE

Nathan Palpant, Lil Pabon, Jeremy Rabinowitz, Cristi Stoick-Cooper, Sharon Paige, Randall Moon, Charles Murry
University of Washington, Seattle, WA, United States

Genetic regulation of the cell fate transition from lateral plate mesoderm to the cardiovascular progenitor cell (CVP) requires suppression of Wnt/β-catenin signaling, but the mechanism for this is not well understood. By analyzing gene expression and chromatin dynamics during directed differentiation of human embryonic stem cells (hESCs), we identified a suppressor of Wnt/β-catenin signaling, transmembrane protein 88 (TMEM88), as a potential regulator of CVP specification. During the transition from mesoderm to the CVP, TMEM88 has a chromatin signature indicative of genes involved in mediating cell fate decisions, and its expression is highly up-regulated prior to expression of key cardiac transcription factors such as Nkx2.5, Isl1, and Tbx5. In early zebrafish embryos tmem88a is expressed exclusively in the bilateral heart fields, and morpholino-mediated reduction in its expression yields small hearts with defective looping. Similarly, short hairpin RNA targeting TMEM88 during hESC cardiac differenti-

ation resulted in reduced expression of genes required for CVP development concommitant with an elevation of factors associated with hemogenic-endothelial development. Analysis of cell differentiation showed that TMEM88 knockdown inhibits cardiomyocyte differentiation and promotes endothelial differentiation. Thus, TMEM88 is indispensable for heart development and acts in the pre-cardiac mesoderm to specify lineage commitment of the cardiovascular progenitor cell through inhibition of Wnt /ß-catenin signaling.

F-3133

MYOSIN PHOSPHATASE MODULATES CARDIAC CELL FATE BY REGULATING THE SUBCELLULAR LOCALIZATION OF NKX2.5 IN A WNT/ROCK-DEPENDENT PATHWAY IN EMBRYONIC STEM CELLS

Ilona Skerjanc[1], Michael Shelton[1], Jean Philippe Lambert[1], Barbora Malecova[2], Sophie Boisvenue[1], Marc Ruel[3], Daniel Figeys[1], Lorenzo Puri[2], Tammy Ryan[1]

[1]University of Ottawa, Ottawa, ON, Canada, [2]Sandford-Burnham Medical Research Institute, San Diego, CA, United States, [3]University of Ottawa Heart Institute, Ottawa, ON, Canada

Nkx2.5 is a transcription factor that regulates cardiomyogenesis in vivo and in embryonic stem cells. It is also a common target in congenital heart disease. Although Nkx2.5 has been implicated in the regulation of many cellular processes that ultimately contribute to cardiomyogenesis and morphogenesis of the mature heart, relatively little is known about how it is regulated at a functional level. We have undertaken a proteomic screen to identify novel binding partners of Nkx2.5 during cardiomyogenic differentiation in an effort to better understand the regulation of its transcriptional activity. Purification of Nkx2.5 from differentiating cells identified the myosin phosphatase subunits PP1β and Mypt1 as novel binding partners. The interaction with PP1β/Mypt1 resulted in exclusion of Nkx2.5 from the nucleus and consequently, inhibition of its transcriptional activity. Exclusion of Nkx2.5 was inhibited by treatment with LeptomycinB and was dependent on a Mypt1 nuclear export signal. Furthermore, in transient transfection experiments, Nkx2.5 co-localized outside the nucleus with phosphorylated Mypt1 in a manner dependent on Wnt signalling and Rho-associated protein kinase. Treatment of differentiating mouse embryonic stem cells with Wnt3a resulted in enhanced phosphorylation of endogenous Mypt1, increased nuclear exclusion of endogenous Nkx2.5 and a failure to undergo terminal cardiomyogenesis. Finally, knockdown of Mypt1 resulted in rescue of Wnt3a-mediated inhibition of cardiomyogenesis, indicating that Mypt1 is required for this process. Thus, we have identified a novel interaction between Nkx2.5 and myosin phosphatase. Promoting this interaction represents a novel mechanism whereby Wnt3a regulates Nkx2.5 and inhibits cardiomyogenesis.

F-3134

CARDIOMYOCYTE CLUSTERS DERIVED FROM MATRIX-FREE GROWTH ADAPTED HUMAN EMBRYONIC STEM CELLS

Narmin Bigdeli[1], Daniella Steel[2], Marianne Jonsson[1], Julia Asp[1], Peter Sartipy[2], Anders Lindahl[1]

[1]Department of Clinical Chemistry and Transfusion Medicine, Institute of Biomedicine, Gothenburg, Sweden, [2]Cellectis Stem Cell, Gothenburg, Sweden

Human embryonic stem cells (hESCs) are
pluripotent cells with great importance for future cell-based therapies.
Despite the recent advances in culture techniques for undifferentiated hESCs,
there is a great need for further improvements until hESCs can be applied to
human medical conditions. Since hESCs are traditionally cultured on
feeder-cells or a coating replacing feeder-cells, some of the issues to address
are a less laborious system, cost-effectiveness, culture stability,
well-defined components, xeno-free culture conditions and compatibility with good
manufacturing practice. In our previous study we presented a novel protocol for
the adaptation of hESC lines which can be cultured directly on plastic surfaces
without any supportive coating i.e. avoiding

feeder-cells and coating matrix. This promotes stable culture condition
of hESCs and facilitates large-scale production, making expansion of hESC less
laborious and time-consuming. This improved culture technique of hESCs which supports the undifferentiated state of the cells is denoted matrix-free growth-hESCs (MFG-hESCs).
Here we report that in vitro differentiation of
MFG-hESCs toward spontaneously beating cardiomyocytes can be achieved through three-dimensional-aggregate formation using well known protocol for cardiomyocytes derivation. In vitro
characterization of beating cardiomyocyte clusters was preformed based on contraction ability of the
cells, gene expression analysis, beat frequency and studying the human Ether-à-go-go-Related Gene (hERG) and I_f ion channels using hERG blocker E4031 and Zatebradine. The result verifies that these spontaneously beating cardiomyocyte clusters share some qualities that are characteristic for
human heart tissue.
Cardiomyocyte cluster derivation manifests the
pluripotency of MFG-hESCs retaining the characteristics of undifferentiated
hESCs and opens the possibility of using non-feeder/coating dependent hESCs as
human in vitro model system in drug discovery process.

F-3135

DEVELOPMENT OF A MOUSE ESC REPORTER LINE TO ISOLATE CARDIAC CONDUCTION SYSTEM CELLS

Fiona See[1], Jia Lu[1], **Karen Maass**[1], Sunita D'Souza[2], Glenn I. Fishman[1]
[1]NYU School of Medicine, NYC, NY, United States, [2]Mount Sinai School of Medicine, NYC, NY, United States

Background: We have previously demonstrated that the cell adhesion protein contactin2 (Cntn2) is enriched in Purkinje cells of the cardiac conduction system (CCS).
Objective: Generation of a mouse embryonic stem cell (mESC) reporter line that allows identification of Purkinje-like cardiomyocytes in vitro.
Methods and Results: mESC were generated from transgenic mice carrying a BAC Cntn2-eGFP reporter gene and were subsequently transduced with lentivirus coding for a selectable MHCα-mCherry cardiomyocyte reporter gene. Immunostaining analysis confirmed that mESC expressed markers of pluripotency (Oct3/4; Klf4) and spontaneously differentiated into cells of all three germ layers in the absence of LIF (α-smooth muscle actin; β-tubulin; α-fetoprotein). Spontaneous or serum-free directed cardiac differentiation resulted in generation of double positive, spontaneously beating cardiomyocytes after three weeks. Yield of double positive cells could be increased by adding endocardial derived factors (Nrg1; ET-1). FACS isolated double positive cells were enriched in transcripts of cardiomyocytes (MLC2v) and the CCS (Cntn2; Cx40). Action potential recordings of eGFP positive cardiomyocytes demonstrated distinct plateau phase and elongated action potential duration (APD50=79.9±10.4ms, APD90=170.2±17.5ms; n=11) compared with eGFP negative cardiomyocytes (APD50=53.4±9.4ms, APD90=120.6±17.3ms; n=15).
Conclusion: We have established a mESC reporter line for the identification of CCS-like cells. This model should be useful for downstream studies of CCS development and pathology. Cntn2 may also be a useful marker of CCS-like cells derived from human ES and/or iPS cells.

F-3136

DATA-FUSED METRICS FOR STEM CELL-DERIVED MYOCYTE QUALITY ASSESSMENT

Sean P. Sheehy, Francesco Pasqualini, Anna Grosberg, Sung Jin Park, Yvonne Aratyn-Schaus, Kevin Kit Parker
SEAS, Harvard University, Cambridge, MA, United States

Advances in stem cell bioprocessing methods and technologies have made commercialization of stem cell-derived cardiac myocytes for *in vitro* research applications practical. While quality assurance metrics exist for safety issues in the manufacture of stem cell-based products, no standardized guidelines currently exist for validating stem

cell-derived myocyte functionality. Traditionally, myocardial differentiation is evaluated using methodologies that provide poor predictions of tissue-level cooperative function and little indication as to how closely stem cell-derived myocytes mimic native cardiac myocytes. We hypothesized that the differentiation state of stem cell-derived myocytes could be more accurately assessed by performing a combination of quantitative measurements of cardiac form and function on engineered tissues fabricated from these cells, followed by computational analysis that integrates and compares them against measurements taken on myocytes with a confirmed cardiac phenotype. To test this hypothesis, we subjected engineered cardiac tissues comprised of either neonatal mouse ventricular myocytes (NMVM), murine ES-derived myocytes (mES), or murine iPS-derived myocytes (miPS) to quantitative cytoskeletal structure analysis, assessment of contractile performance, electrophysiological recordings, and gene expression measurements and evaluated the results using the NMVM tissues to represent the target phenotype. Examination of global sarcomere alignment as judged from immunohistochemically-labeled α-actinin micrographs revealed that the mES and miPS tissues possessed reduced sarcomere alignment due to the presence of less mature myofibrils relative to the NMVM tissues. Measurement of contractile stress output using our muscular thin film assay revealed that both the mES and miPS tissues generated significantly less contractile stress than the NMVM tissues. Optical mapping studies showed that tissues constructed from all three myocyte types exhibited indistinguishable conduction velocities, but the action potential duration at 90% of the amplitude (APD90) for mES tissues was significantly shorter than that observed in NMVM tissues, whereas APD90 in miPS tissues was not significantly different from either the mES or NMVM tissues. Statistical comparison of phenotype marker genes commonly used to distinguish ventricular from atrial myocytes revealed that the mES and miPS samples exhibit significantly greater expression of some atrial markers (*Myl4, Tbx5, Hcn4*) and significantly lower expression of some ventricular markers (*Pln, Irx4, Kcne1*) than the NMVM tissues. Together, these measurements provide a complementary overview of the phenotypic state of cardiac tissues that is more informative than the output of any assay alone, and may be integrated using machine learning to create a stem cell "Quality Index" for validating the output of bioprocess protocols.

F-3137

IDENTIFICATION OF PROTEOMIC ALTERATIONS ASSOCIATED WITH THE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO CARDIOMYOCYTES BY SILAC

Sarah A. Konze[1], Johannes Zeiser[2], Andreas Pich[2], Robert Zweigerdt[3], Falk F. Buettner[1]

[A]Institute for Cellular Chemistry, [B]Institute of Toxicology, [C]Leibniz Research Laboratories for Biotechnology and Artificial Organs / HTTG, [1]Hannover Medical School, Hannover, Germany

Human pluripotent stem cells, both embryonic (hESCs) and induced (hiPSCs), are promising tools for regenerative therapies. For instance, they can be differentiated into cardiomyocytes and might be used to replace infarcted heart tissue in the future. However, for transplantation purposes it is necessary to deplete the generated cardiomyocytes of other cell types in order to avoid complications such as teratoma formation caused by remaining pluripotent stem cells.

Purification of stem-cell-derived cardiomyocytes is still hampered by a lack of specific cell surface markers. Only recently, discovery of the cell surface protein SIRPα as cardiomyocyte-specific has shed light onto this problem, but a future therapeutic application would require a reliable set of several markers for both positive and negative selection.

This study is focused on the identification of novel cardiomyocyte-specific proteins applying a global quantitative proteomic approach by mass spectrometry using an LTQ Orbitrap Velos. We performed a stable isotope labeling in cell culture (SILAC)-based comparison of both hESCs and hiPSCs with stem-cell-derived embryoid bodies containing cardiomyocytes. These were generated via an established chemically-defined serum-free differentiation protocol. In addition, genetically modified hESCs carrying a neomycin resistance under the control of the cardiomyocyte-specific α-MHC promoter were used to generate pure cardiomyocyte populations by antibiotic selection. hESCs and hiPSCs were labeled during culture on mitotically inactivated human fibroblasts and by determining the portion of SILAC labeled proteins of fibroblast origin we were able to subtract the impact of the fibroblasts on labeling efficiency.

The proteomic analysis identified over 2000 proteins with approximately 140 proteins being significantly up- or downregulated upon differentiation to cardiomyocytes, respectively. Using the open access protein-protein interaction database STRING 9.0, gene ontology analysis was performed with these significantly regulated proteins in order to gain information about differentially regulated biological pathways and especially about the cellular localization of the regulated proteins. Candidate proteins classified as plasma membrane associated were further scrutinized regarding their cell-type specific expression in order to identify novel cell surface markers for pluripotent stem cells and stem cell-derived cardiomyocytes.

F-3138

CARDIOTOXICITY ASSESSMENT OF ANTI-CANCER KINASE INHIBITORS USING HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTE BASED ASSAYS

Hai-Qing Xian[1], Carmina Blanco[1], Mathew Brock[2], Ralph Snodgrass[1]

[1]VistaGen Therapeutics, Inc., South San Francisco, CA, United States, [2]Axion Biosystems, Atlanta, GA, United States

The majority of drug candidates in development are targeted for cancer therapy. Small molecule kinase inhibitors (KIs) belong to a new category of drugs that have revolutionized cancer therapy due to decreased systemic toxicity and increased target cell efficacy compared to classic cancer drugs. However, many KIs have been implicated in causing serious adverse cardiac events in patients, which were not identified during preclinical drug development. Therefore, development of sensitive assays for early detection of cardiotoxicity is critical to help avoid adverse patient effects, late-stage clinical termination, or recall from the market. Our goal is to utilize human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) to develop reproducible and reliable assays for the prediction of drug-induced cardiomyopathy and/or arrhythmogenesis. Using our optimized differentiation protocol, which does not necessitate selection, human PSC cells can be differentiated to cultures containing 80-96% cardiomyocytes that function reliably in various established and newly developed assays relevant to cardiac drug effects. Utilizing these high purity hPSC-CMs, we have developed several high-throughput cell-based assays for assessment of drug-induced cardiac cytotoxicity that results from multiple mechanisms, including apoptosis, mitochondrial toxicity, oxidative stress, and energy metabolism disruption. Here we demonstrate the utility of this system to detect cardiac toxicities of well-known anti-cancer kinase inhibitors, including imatinib, dasatinib, sunitinib, erlotinib and temsirolimus, which have been associated with adverse clinical cardiac events that were not detected during the drug development process. The data indicate that our hPSC-CM based assays successfully detect cardiotoxicity induced by representative compounds from different KI categories, which primarily target Bcr-Abl, IRMs, VEGFRs, EGFR, PI3K, MEK or mTOR. Additionally, the assays provide clues to the mechanism of cardiac cytotoxicity induced by each compound. Further characterization of the functional effects induced by the KI inhibitors were evaluated via a 48-well format multi-electrode array (MEA) system. The data indicated that sunitinib and dasatinib caused QT prolongation, arrhythmia, and/or altered contraction rates in hPSC-CMs, which is consistent with clinical observations. In addition, no cardiac toxicity or alteration in electrophysiology was detected with drugs that do not have a cardiac liability, emphasizing the reliability of our assay system. To conclude, our data suggest that this hPSC-CM based high throughput screening system will be a clinically predictive system for preclinical cardiac safety screening of anti-cancer and other therapeutic compounds, helping not only to identify toxicities, but also to discover potential mechanisms of action.

F-3141

DEVELOPMENT OF FUNCTIONAL HUMAN CELL BASED CARDIOVASCULAR CONSTRUCT

Kirsi Kujala[1], Hanna Vuorenpää[2], Jertta-Riina Sarkanen[2], Mari Pekkanen-Mattila[1], Timo Ylikomi[2], Tuula Heinonen[2], Katriina Aalto-Setälä[1]

[A]BioMediTech, Institute of Biomedical Technology, [B]Finnish Centre for Alternative Methods, [1]University of Tampere, Tampere, Finland

With human embryonic stem cell (hESC) -derived cardiomyocytes (CM) it is possible to generate cardiac tissue constructs which can improve cardiotoxicity testing and drug discovery and safety. In cardiac tissue engineering applications, in vitro construct should also include vascular structures as it is present in heart. Here the aim was to develop 2D in vitro cardiovascular construct modeling human heart.
In the construct hESC derived CMs were co-cultured on top of a supporting vascular-like network. The vascular-like network formed by human adipose stromal cells (hASCs) or human foreskin fibroblasts and human umbilical vein endothelial cells (HUVECs) served as a natural scaffold in the cardiovascular construct. Tubule formation, cellular orientation, maturation, functionality and drug responses of this cardiovascular construct were characterized.
Preliminary results show that CMs were elongated and aligned with the vascular-like network and formed a synchronously beating cell construct. The electrical activity as well as calcium metabolism was shown to be normal in this construct as well as response to different drugs.
In conclusion, vascular-like network support the growth of human derived CMs and their contractile properties. When combining vascular-like network with hESC derived CMs the morphology as well as functionality of this cardiovascular construct was improved. Our results suggest that this construct can serve as a model which can be exposed to chemical compounds targeting human cardiovascular system.

F-3142

HEMATOPOIETIC CELLS ARE REQUIRED FOR PROPER DEVELOPMENT OF CORONARY VASCULATURE

Gentian Lluri[1], Xiaoqian Liu[2], Atsushi Nakano[2]
[A]Medicine, Section of Cardiology, [B]Molecular Cell and Developmental Biology, [1]University of California, Los Angeles, Los Angeles, CA, United States

Objective: We examined whether the hematopoietic cells induce the coronary artery formation using genetically modified mouse models of hematopoietic ablation in vivo and ex vivo.
Methods: As a model of for hematopoietic cell deficient animals, we used Runx1 (a transcription factor required for definitive hematopoiesis) knockout embryos and Vav1-cre; R26-DTA embryos, which ablates 2/3 of CD45+ hematopoietic cells. The coronary growth and the hematopoietic cells were evaluated in whole-mount, section and ex vivo explant culture.
Results: The developing coronary endothelial cells form blood-island-like structure at around E12.5 in the subepicardial region. Interestingly, however, the histological analyses suggest that the first Ter119+ and CD45+ blood cells appear in the subendocardial area at E10.5, even before the formation of coronary channels. These initial hematopoietic cells in the heart are not likely derived from the epicardium, as the sorted epicardial cells yielded no hematopoietic cell in colony formation assay. These observations raised a question whether these heart-resident hematopoietic cells rather play an inductive role during coronary formation. To examine this possibility, we analyzed two hematopoietic ablation models. Both Runx1 knockout embryos and Vav1-cre; R26-DTA embryos revealed disorganized, hypoplastic microvasculature of coronary vessels on section and whole-mount stainings. Furthermore, coronary explant experiments showed that the mouse heart explants from Runx1 knockout embryos and Vav1-cre; R26-DTA embryos exhibited impaired coronary formation ex vivo.
Conclusion: Hematopoietic cells are not merely transported via coronary vessels, but substantially involved in the induction of the coronary vessels during cardiogenesis.

F-3143

SYSTEMATIC DEVELOPMENT OF CYTOPROTECTIVE STRATEGY USING COMBINATORIAL PRECONDITIONING OF STEM CELLS TO REDUCE ISCHEMIA REPERFUSION INDUCED APOPTOSIS

Kshitiz Kshitiz[1], David D. Ellison[3], Junaid Afzal[4], Maimon E. Hubbi[4], Hee-Seok Yang[1], Jinah Jang[1], Jatinder Goyal[5], Eun Ahn[2], Segun Bernard[6], Connie Chang[3], Roselle M. Abraham[4], Andre Levchenko[7], Deok-Ho Kim[1]

[A]Bioengineering, [B]Oncology, [1]University of Washington, Seattle, WA, United States, [A]Biomedical Engineering, [B]Medicine, [C]Urology, [D]Radiology, [2]Johns Hopkins Medical Institutions, Baltimore, MD, United States, [3]Biomedical Engineering, Johns Hopkins University, Baltimore, MD, United States

Myocardial infarction (MI) is the leading cause of death and morbidity in the developed world. MI causes significant loss of cardiomyocytes resulting in impaired heart function. Cardiac regeneration using cell therapy has great potential to revolutionize heart failure treatment. In particular, autologous cardiosphere derived cells (CDCs) obtained from percutaneous endomyocardial biopsies have been demonstrated to enhance global and local heart function in human patients. However, most stem cell transplantation strategies suffer from low cell retention, massive cell death, and low long term survival rate. Though efficient cell retention is desirable for any cell type, for autologous cell types it presents a particular hurdle since it increases the interval between initial biopsy preparation and therapy. Ensued delay can result in increased patient discomfort and reduced tissue amelioration. Several factors could play a role in cell death, including damage during isolation and injection, hypoxia, reperfusion, absence of survival factors, disruption of cell-cell and cell-ECM contacts and inflammatory cytokines. In particular, ischemia causing anoxia or hypoxia results in production of cyto-toxic superoxides and peroxide upon reperfusion which is fatal to many stem cell types.
Here, we propose to systematically investigate the pro-survival/anti-apoptotic factors in CDCs using high throughput protein printing technology on bioengineered substrates that allow us to print proteins, lipids, and other biomolecules for large scale screens. Using high-throughput protein printing screen and systematic combinatorial development path of a cytoprotective cocktail, we propose a comprehensive strategy for cell transplantation to prevent cell death due to reperfusion insult. This comprehensive cocktail is composed of a combination of minimal number of cytoprotective biochemical ingredients, hypoxic preconditioning and providing physiological cell environment to cells. Our cocktail resulted in >90% decrease in cell death in vitro, and >5 times increased cell retention in a rat model of MI and reperfusion when measured using bioluminescence imaging. Biochemical analysis indicated that the cocktail presents greater cytoprotection than individual saturated ingredients by inhibiting the apoptosis pathway at multiple junctions, thereby providing fewer avenues for cell to die in response to peroxide insult. Interestingly, the cocktail contained the paracrine factors released by CDCs that are ascribed to their healing potential. We conjugate the ingredients in cocktail with bioengineered heparin-conjugated fibrin allowing for controlled release to provide sustained cytoprotection to the transplanted cells until they integrate within the tissue.
Systematic development of a minimal ingredient cytoprotective cocktail can result in significant reduction in stem cell death post transplantation allowing therapy to be instituted earlier, with fewer cells and potentially higher efficacy.

F-3144

ON-DEMAND PRODUCTION OF VENTRICULAR MYOCYTES FROM HUMAN PLURIPOTENT STEM CELLS

Winston Shim, Ashish Mehta, Yuliansa Sudibyo, Glen Lester Sequiera, Chrishan JA Ramachandra
National Heart Centre Singapore, Singapore, Singapore

Consistent supply of cardiomyocytes is key to maximizing the potential of human pluripotent stem cells (hPSCs) in drug toxicity screening, disease modeling and cardiac regeneration therapy. However, current protocols of cardiac differentiation are limited by lack of reproducbility as well as transferrability between different hPSC lines.
Aim: We describe a uniform protocol for efficient ventricular myocyte production consistently across multiple hPSC lines.
Methods: Gene expression arrays and flow cytometry were utilized to map the temporal prolife of differentiation to characterize cardiac subtype derivation during directed differentiation.
Results: Our serum-free embryoid body (EB)-based protocol was verified on hESC, normal and disease-specific hiPSC lines (total of 8 lines). All cell lines displayed a high degree of contraction (90%) by day 12-13 of differentiation. Kinetic quantitative gene expression profiling (day 0, 1, 2, 4, 6, 8, 14 and 18) of 50 developmental cardiac markers expressed at different stages were evaluated. Gene expression profiling showed sequential upregulation of early mesodermal (brachyury) and cardiac mesodermal (Isl1) markers, cardiac committed markers (GATA4, NKX2.5)

and followed by cardiac specific markers (MLC2a, MLC2v, cTnI, MYH7). A significant upregulation of MLC2a was observed as early as day 4 of differentiation. However, ventricular marker, MLC2v was seen only by day 14 onwards along with cardiac troponin expression. Further, expression of HCN2/4 (nodal markers) was not seen till day 18 of differentiation. Flow cytomertic analysis revealed co-expression of SIRPA and NKx2.5 in 70-80% EBs on day 8, whereas 70-90% expressed cardiac troponin I (cTnI) on day 18, of which 98% of cTnI positive cells were also positive for MLC2v.
Conclusion: We describe a simple off-the-shelf protocol that facilitates on-demand production of large quantities of ventricular biased cardiomyocytes, in a cell-line insensitive manner, which is crucial in translating regenerative potential of hPSCs.

F-3145

DEVELOPMENT OF IN VITRO EFFICACY SCREENING TEST METHODS USING EMBRYONIC STEM CELLS FOR GENE THERAPY PRODUCTS ON ISCHEMIC HEART DISEASE

Ki Dae Park, Soyeong Kang, Sung Yeol Han, Ju Hyun Choi, Sang-Kil Lee, Sun-Young Baek, Il Ung Oh, Joon-Ho Eom, Sun-Hee Kim, Minjung Choi, Si Hyeong Yoo
National Institute of Food and Drug Safety Evaluation, chungcheongbuk-do, Korea, Republic of

Embryonic stem (ES) cells give rise to the possibility to introduce the stem cell therapy for regenerative medicine, even in the field of drug discovery such as biotherapeutics. There is an urgent need for relevant *in vitro* assays that make use of ES cells because of their rich biological utility. In this study, we intended to develop *in vitro* test for efficacy evaluation of gene therapy product. First, we intended to established gene therapy products for this study. We investigated the gene candidates for treatment of ischemic heart disease with the international clinical data and Pub-med. And then VEGF and GATA-4 genes were selected for gene therapy products and DNA plasmid was accepted for Vector system. Thereafter, we induced cardiac differentiation from ES cells with Embryonic Stem Cell Test (EST) method using LIF. Because the rate of functional differentiation of EST method was too high to evaluate for efficacy of gene therapy product on Ischemic Heart Disease, we modified protocol using serial concentration of LIF for relevant cardiac differentiation. We observed that this system revealed VEGF and GATA-4 gene therapy products increased the functional cardiac differentiation and expression of TNNT. These *in vitro* assay results validated with *in vivo* Ischemic Heart Disease animal models. Taken together, we studied for establishment of foundation as *in vitro* test using ES cells for evaluation of ischemic heart disease gene therapy products.

F-3146

ENHANCED GRAFT SUCCESS AFTER MYOCARDIAL INFARCTION FOR ADULT RAT CARDIAC STEM/PROGENITOR CELLS PRIMED WITH FACTORS SECRETED BY ADULT HUMAN STROMAL PROGENITORS (p75MSCs).

Krithika Rao, Yoshitaka Iso, Jeffrey L. Spees
Medicine, University of Vermont, Colchester, VT, United States

Effective stem/progenitor cell therapy for regenerative medicine depends largely on initial graft success with donor cells. After transplantation, cells have to successfully adhere, migrate, differentiate, and functionally integrate into host tissue. However, cultured adult stem/progenitor cells, embryonic stem cell derivatives and induced pluripotent stem cell derivatives often survive poorly after transplantation. This is especially true after grafting to tissues injured by ischemia, such as the heart after myocardial infarction (MI). We focused on strategies to enhance graft success with adult cardiac stem/progenitor cells, administered 24 hours after ischemic injury to the heart. Stromal cells have been reported to support the growth of stem/progenitor cells in culture and contribute to tissue repair in vivo, in part, by secreting a milieu of growth/reparative factors. Notably, some stromal cell-secreted factors are reported to stimulate proliferation of resident endogenous stem/progenitor cells. We thus hypothesized that priming cells with a cocktail of stromal cell-secreted ligands would promote graft success for adult rat cardiac stem/progenitor cells (CSCs/CPCs) after myocardial infarction. Conditioned medium (CdM) was generated from adult human bone marrow derived multipotent stromal cells that were isolated by plastic adherence (typical MSCs) or magnetic

sorting against the p75 low-affinity nerve growth factor receptor (p75MSCs). CdM supported adult rat CPC proliferation under normoxia and survival under hypoxia (1% oxygen). CPCs retained their multipotency after incubation in CdM. GFP-positive CSCs primed in 30x p75MSC CdM for 30 min demonstrated robust sub-epicardial engraftment and migration into host tissue compared with vehicle-primed cells. Lineage tracing demonstrated that GFP-positive CSC derivatives migrated into zones with infarction and expressed markers of endothelial cells, smooth muscle cells, and myofibroblasts. Furthermore, they contributed to repair of damaged blood vessels at 1 week after MI and transplantation. At 1 month post MI, engrafted cells populated the majority of the infarcted tissue in the heart. Differentiated cells contributed to the repair of existing blood vessels and many vessels appeared to consist mostly of GFP-positive cells. Microarray assays of human p75MSCs were used to identify candidate secreted factors. We then performed screens based on antibody-mediated neutralization and CPC protection assays under hypoxic conditions. We identified 2 soluble factors present in CdM that acted synergistically to protect CPCs under hypoxia. Furthermore, a defined ratio of these two factors based on that found in CdM was capable of effectively priming CSCs, markedly promoting graft success after MI.

F-3147

ROBUST CARDIAC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS UNDER DEFINED, CYTOKINE FREE AND XENO FREE CONDITIONS

Itsunari Minami, Kazuhiro Aiba, Motonari Uesugi, Norio Nakatsuji
iCeMS, Kyoto Univ, Kyoto, Japan

Human embryonic stem cells and induced pluripotent stem cells (ES/iPSCs), are potentially useful in regenerative therapies for heart disease. For medical applications, clinical grade cardiac cells must be produced from ES/iPSCs in a defined, cost-effective manner. KY02111, a novel small molecule, promotes differentiation of ES/iPSCs to cardiomyocytes. Although the direct target of KY02111 remains unknown, results of the present study suggest that KY02111 promotes cardiac differentiation from ES/iPSCs in a manner that is distinct from previously studied small molecules. Combined use of KY02111 and several other small molecules produced robust cardiac differentiation of ES/iPSCs in a xeno-free, defined medium, devoid of serum and any kind of cytokines, such as BMP4, Activin A or WNT3A. The methodology has potential as a means for the practical production of human cardiomyocytes for regeneration therapies.

F-3151

CHARACTERIZATION OF CARDIAC LINEAGES USING INTRACELLULAR AND SURFACE IMMUNOPHENOTYPING BY FLOW CYTOMETRY

Jody C. Martin[1], Jennifer Lowe[2], Christian T. Carson[1], Ju Chen[2], Sylvia Evans[2]
[1]BD Biosciences, San Diego, CA, United States, [2]University of California San Diego, La Jolla, CA, United States

Cardiac differentiation from human pluripotent stem cells often yields inconsistent and heterogeneous populations that are problematic for quantitative and comparative analyses. To solve this problem, we used intracellular immunostaining in combination with cell surface markers for prospective identification of naive and mature cardiomyocytes, as well as other lineages. We used multicolor Flow Cytometry to quantitatively characterize distinct batches of hESC derived cardiac differentiations. From this analysis at multiple time points during differentiation, we defined specific cell populations that characterize the efficiency and heterogeneity of individual differentiations. This method is ideal for iteratively improving differentiation protocols. Additionally, cell sub-populations can be purified by fluorescence-activated cell sorting (FACS), which will be useful for downstream analyses such as FISH, microarray analysis, and miRNA analysis.

F-3152

GLUCOCORTICOID SIGNALING PROMOTES CARDIOGENESIS IN ESC THROUGH AN HNF4A-CER1 CASCADE.

Erik Willems[1], Joaquim Cabral-Teixeira[1], Wenqing Cai[1], Almudena Martinez-Fernandez[2], Andre Terzic[2], Mark Mercola[1]

[1]Muscle Development and Regeneration Program, Sanford-Burnham Medical Research Institute, La Jolla, CA, United States, [2]Cardiovascular Diseases, Mayo Clinic, Rochester, MN, United States

The anterior visceral endoderm (AVE) was established as a source of cardiac inducing signals in the mouse embryo in the late nineties and sequential reports have attributed the cardiac activity to the expression of Wnt antagonists such as Dkk1. Little is known, however, about how these factors are regulated to control cardiac fate. Through a screen of active biologics in embryonic stem cells (ESC), we have identified Dexamethasone (Dex), a synthetic glucocorticoid, as a potent inducer of cardiac fate at the time that mesoderm is specified. Since the glucocorticoid receptor (GR) is expressed in the AVE in mammals, we hypothesized that GR signaling may regulate cardiac fate in ESC via modulation of AVE signals during the specification of the mesoderm to the cardiac lineage.
We demonstrated that Dex was indeed most active in inducing cardiac differentiation when mesoderm cells emerge and siRNA knock-down of GR in presence of Dex indicated that Dex was indeed mediating this pro-cardiac effect through GR signaling. To understand how GR signaling was driving cardiac fate, we probed untreated and Dex-treated cultures for the expression of markers representing the different germ layers in the gastrulating embryo. Dex induced the cardiovascular progenitor markers Mesp1 and Pdgfra, as expected, yet no other mesoderm, definitive endoderm or neural markers were affected, indicating Dex is driving cardiac specification from uncommitted mesoderm progenitors. Endothelial cells and smooth muscle markers were also increased further indicating GR is targeting a common cardiovascular progenitor. Since GR is expressed in the AVE, we also probed markers for this pro-cardiac tissue and found that Dex selectively enhanced expression levels of HNF4a and Cer1. Knock-down experiments placed these factors downstream of GR and demonstrated that HNF4a and Cer1 are essential to the cardiac induction program initiated by Dex and executed by Cer1. Cer1 is a secreted growth factor known to drive stem cell cardiogenesis.
We thus describe a novel essential cascade of factors controlled by GR signaling to drive the cardiac differentiation program in ESC cultures, and which most likely also contributes to the development of the mammalian heart. Moreover, the effects of GR signaling on cardiac progenitors in ESC may shed some light on how adult cardiac progenitors may be triggered to regenerate the heart after infarct.

F-3153

PREDICTIVE HIGH-THROUGHPUT ASSAYS FOR HEPATOTOXICITY AND CARDIAC PHYSIOLOGY USING INDUCED PLURIPOTENT STEM CELL (IPSC)-DERIVED CELL MODELS

Oksana Sirenko[1], Jayne Hesley[1], Carole Crittenden[1], Vanessa Ott[2], Blake Anson[2], Evan F. Cromwell[1]

[1]Molecular Devices, Sunnyvale, CA, United States, [2]Cellular Dynamics International, Madison, WI, United States

Development of highly predictive in vitro assays is extremely important for improving the drug development process and reducing drug attrition due to toxicity. iPSC-derived cell models are rapidly being adopted by the pharmaceutical industry for pre-clinical toxicity studies. iPSC-derived cells provide relevant human biology and are increasingly being shown to accurately predict drug-induced toxicity. Human iPSC-derived hepatocytes, cardiomyocytes or neurons show great promise with respect to primary tissue-like phenotype, unlimited availability, and a potential to establish cells from different individuals. To enable the full potential of iPSC-derived cell models, it is necessary to develop highly predictive in vitro assays that can be performed in a high throughput manner. We have developed several assays to assess drug-induced hepatotoxicity and cardiotoxicity.
First, we used high content imaging and iPSC-derived hepatocytes to assess general and mechanism-specific toxicity. A library of 240 known hepatotoxic compounds was analyzed over a range of concentrations using automated multi-parametric image analysis on a cell-by-cell basis. The endpoints assessed were cell viability, nuclear shape, average and integrated cell area, mitochondrial membrane potential, accumulation of phospholipids, cytoskeleton integrity, and apoptosis. We found that multi-parametric automated image analysis greatly increases assay sensitivity while also providing important information about toxicity mechanisms. Specifically, we found that multi-paramet-

er assessment increased sensitivity of the assay to 60% (with 92% specificity). In addition, the assay also demonstrated high sensitivity (70%) for selected classes of compounds such as neuroleptic, cardiac, anti-fungal, anti-cancer drugs.
In addition to end-point toxicity assessment by high content imaging we have developed a physiological assay for measuring the impact of pharmacological compounds on the beating rate of human iPSC-derived cardiomyocytes with fast kinetic fluorescence imaging systems. Cardiomyocyte contraction rate and pattern was characterized by monitoring changes in intracellular Ca^{2+} measured using calcium sensitive dyes. The assay allows automated characterization of deviations from normal beating rate, peak width, or waveform irregularities. We have validated the assay system with a library of cardiotoxic compounds consisting of 131 compounds representing different classes of anti-arrhythmia drugs, receptor antagonists, ion channel blockers, and kinase inhibitors. The estimated predictive value of the multi-parametric assay was greater than 80% (with 86% sensitivity and 93% specificity). The multi-parametric analysis has also demonstrated great value in predicting long QT syndrome, and other types of arrhythmic and non-arrhythmic cardiotoxicity.
We conclude that these high-throughput, high-content automated screening assays using iPSC-derived hepatocytes or cardiomyocytes are feasible, reproducible, have high predictive value and correlation with known *in vivo* toxicity, and can facilitate safety assessment or drugs and chemicals.

F-3154

DIRECT EPIGENETIC CARDIAC TRANSDIFFERENTIATION OF HUMAN AMNIOTIC MESENCHYMAL STEM CELLS

Yuka Matsuura, Xiaohu Ge, Il Suk Sohn, Scott A. Metzler, Paul J. Kim, Rajesh Dash, Phillip C. Yang
Department of Medicine, Stanford University School of Medicine, Stanford, CA, United States

Background: The human amniotic mesenchymal stem cells (hAMSCs) demonstrate partially pluripotent and precardiac characteristics. High native expression of pluripotency genes including, Oct 4 and Nanog, and robust stemness expression of SSEA3, SSEA4, and Tra1-81 cell surface markers are observed. Furthermore, early cardiac developmental genes including Mesp1, GATA4, and Nkx2.5 are found. Epigenetic reprogramming by valproic acid (VPA), a histone deacetylase inhibitor, induces ntermediate pluripotency in hAMSCs. This pluripotency state provides an epigenetically unstable environment to allow direct transdifferentiation while bypassing the full pluripotency state. Specifically, the hAMSCs are dedifferentiated into precardiac mesodermal state and undergo direct transdifferentiation into cardiac progenitor-like cells, using Wnt-pathway specific small molecules. In this study, we examined the feasibility of modifying the pluripotency state of hAMSCs and their subsequent direct cardiac transdifferentiation potential. Methods: The hAMSCs were enzymatically digested from human placenta amniotic membrane and cultured in hAMSC media. On reaching 80% confluence, the media was changed to human embryonic stem cell (hESC) culture condition with 0.5, 1.0, 1.5, 3.0 and 6.0mM of VPA (Sigma-Aldrich, Missouri). The hAMSCs treated by 1.0mM VPA for 5 days were divided into 3 protocols to undergo cardiac transdifferentiation for 6 days and followed for additional 12 days: 1) 6μM CHIR-99021 (Wnt activator, Selleck Chemicals, Texas) and 5μM IWR-1 (Wnt inhibitor, Sigma-Aldrich, Missouri), 2) 12μM CHIR-99021 and 5μM IWR-1 and 3) 6μM CHIR-99021, 2nM BMP4 (R&D Systems, Minnesota) and 5μM IWR-1. Flow cytometry, real time PCR, and immunohistology were performed before and after VPA treatment and during the transdifferentiation process.Results: The hAMSCs underwent robust non-integrative epigenetic reprogramming using VPA in hESC culture condition to generate a dedifferentiated, partially reprogrammed population of hAMSCs, marked by the emergence of 50% Tra1-60 positive cells and significant up-regulation of Nanog, Brachyury, GATA4 and Nkx2.5 genes (5-, 10-, 10- and 10-fold, respectively). The 3 differentiation protocols all increased the expression of the early cardiac (GATA4 and Nkx2.5), mesodermal (Brachyury and Mesp1), and pluripotency (Oct4, Nanog and Rex1) genes. Comparison of the 3 protocols demonstrated that Protocol 3 (6μM CHIR-99021, BMP4 and IWR-1) generated the highest gene expression of GATA4 (550-fold), Nkx2.5 (30-fold), Brachyury (10-fold), Oct4 (5-fold), Nanog (2.5-fold), and Rex1 (10-fold). Confocal microscopy confirmed robust immunohistological expression of the cardiac phenotypes in these cells, demonstrating high expression of Nkx2.5 and cardiac troponin not seen prior to the transdifferentiation. Conclusion: VPA can dedifferentiate the hAMSCs into higher pluripotency state. Sequential administration of Wnt-specific small molecules generated cardi-

ac progenitor-like cells from hAMSCs. Further studies to increase the efficiency of cardiac transdifferentiation and to assess their in vivo cardiac regenerative effects are underway.

F-3155

A REGENERATIVE MEDICINE APPROACH FOR ENDOGENOUS CARDIAC REPAIR: DEVELOPMENT OF A HIGH CONTENT ASSAY FOR EPICARDIAL PROGENITOR CELL PROLIFERATION

Lauren Drowley[1], Anna Jonebring[1], Isabelle Jansson[1], Marie-Jose Goumans[2], Qing-Dong Wang[1]
[1]AstraZeneca, Mölndal, Sweden, [2]Leiden University, Leiden, Netherlands

Post-myocardial infarction cardiac dysfunction and heart failure remain a medical challenge as current therapies do not address the fundamental pathology, i.e. loss of functional cardiomyocytes. Epicardial progenitor cells (EPDCs) have been shown to play a major role in both heart development and repair of the myocardium. They are activated, although insufficiently, in adult hearts after injury and undergo an epithelial-to-mesenchymal transition that is characterized by expression of Wilm's Tumor protein 1 (WT-1). By expanding the number of activated EPDCs in situ, we aim to increase functional repair and regeneration. Here we describe the development and validation of a phenotypic assay for molecules that enhance endogenous regenerative capacity of EPDCs.
EPDCs were characterized for basal levels of proliferation as well as expression of progenitor and differentiation markers, including WT-1, vascular endothelial growth factor receptor, and cardiac troponin T. Cells were expandable to passage 11 without a decrease in WT-1 expression or changes in proliferation capacity, providing further support for the progenitor characteristics of the isolated populations. We tested compounds from the literature that have been shown to have pro-proliferative effects in stem/progenitor cells at various timepoints which allowed us to determine the optimal window where a proliferative increase could be seen above basal levels. In EPDCs, we have demonstrated a significant increase in proliferation after treatment with a combination of PDGF and bFGF while maintaining the expression of WT-1, which has subsequently been used as a positive control.
To enable screening of compounds at high throughput we further developed an endpoint imaging assay where the cells were stained with WT-1 and Ki-67 with Hoescht 33342 as a nuclear counterstain. To test the robustness of the assay, we treated the cells with an expanded set of compounds drawn from the literature including neuregulin-1, periostin, lithium chloride, thymosin β4, and VEGF. After assay validation, we are moving into screening with a larger number of compounds including sets where the mechanism of action is known, which will aid in later deconvolution.
Here we have applied high-content phenotypic screening to assess the proliferation of epicardial progenitor cells following small molecule intervention. This assay has the potential to be used as the basis for proliferation screens in other progenitor cell types.

F-3156

NKX2-5 SUPPRESSES THE FORMATION OF THE CONDUCTION CELLS AND THE PROLIFERATION OF ATRIAL CARDIOMYOCYTES

YASUHIRO NAKASHIMA[1], DIANA A. YANEZ[1], MARLIN TOUMA[2], MARIA C. JORDAN[3], KENNETH P. ROOS[3], ATSUSHI NAKANO[1]
[1]Department of Molecular, Cell and Developmental Biology, UCLA, Los Angeles, CA, United States, [A]Department of Pediatrics & Department of Molecular, Cell and Integrative Physiology, [B]Department of Physiology, [2]David Geffen School of Medicine, Los Angeles, CA, United States

Nkx2-5, a cardiac homeobox transcription factor, plays pivotal roles in multiple steps during the differentiation of the multipotent cardiac progenitors into diverse cardiovascular lineages. Mutation of human *NKX2-5* is linked to atrioventricular conduction abnormalities and atrial septal defects. However, it has been difficult to rigorously examine the primary role of Nkx2-5 during the formation of the cardiac conduction cells in part due to the concurrent hemodynamic aberration associated with ventricular dysfunction. Here, we examined the role of Nkx2-5 in the

formation of the conduction cells in the atria. *Nkx2-5* was conditionally ablated in the atrial cardiomyocytes using atrial-specific *Sln-Cre* mouse line. The conditional mutants died shortly after birth, which is earlier and more severe than ventricular-specific knockout of *Nkx2-5*. While no evidence of ventricular failure was identified, atrial-specific conditional mutants developed congenital heart abnormalities including enlarged foramen ovale and hyperplastic atrial myocardium. Histological analyses revealed that the overproliferation of the atrial myocytes underlies the hyperplastic atrial myocardium prior to the development of the atrial septal enlargement. Moreover, conditional mutants showed massive expansion of the internodal conduction tracts and atrioventricular conduction blocks that were further exacerbated by catecholamine stimulation. Given that the hemodynamic alteration is minimal during the gestational stages in our model, the misspecification of the conduction cells is not likely due to the alteration of the hemodynamics. Together, these data suggest that Nkx2-5 plays a suppressive role during the formation of the internodal conduction cells and the proliferation of the atrial cardiomyocytes.

Eye or Retinal Cells

F-3161

GENOME ENGINEERING TOOLS FOR GENERATING RETINAL AND RPE REPORTERS.

Karl J. Wahlin, Vinod Ranganathan, Valentin Sluch, Catherine Kim, Julien Maruotti, Donald J. Zack
Johns Hopkins School of Medicine, Baltimore, MD, United States

Human stem cells when differentiated into retinal neurons, particularly photoreceptors, offer a convenient means to study not only mechanisms of retinal development but also the pathogenesis of retinal diseases, such as age-related macular degeneration and the photorecptor degeneration retinitis pigmentosa. The development of retinal cell-type specific reporters to monitor cells in vitro would be highly advantageous towards these efforts since they would facilitate efforts to speed up ongoing small molecule screens aimed at identifying pathways that promote retinal development and prevent photoreceptor loss. To generate these reporters we have adapted a solid state TALEN (TALE effector nuclease) assembly method, referred to as iterative capped assembly (ICA), that involves serial ligations of TALE monomers on magnetic beads to synthesize TALENs. We also are adapting a higher throughput alternative involving CRISPR gRNA nucleases, a technology that uses small RNAs to guide the cleavage of DNA by a CRISPR-associated (Cas9) nuclease.
We have synthesized TALENs targeting the early eye-field marker Rax, as well as the early photoreceptor markers Crx and RBP3 (interphotorececptor binding protein). Late stage photoreceptor markers including the visual pigments rhodopsin and cone-opsins are being assembled likewise. CRISPR gRNAs targeting genomic sites close to those TALEN targets have been assembled to compare the relative efficiencies of each system. In addition to the photoreceptor reporter constructs we have also generated gRNAs targeting sites that are preferentially expressed in RPE (retinal pigment epithelium) cells and each of the major neuronal cell types within the inner retina, including bipolar, horizontal, amacrine and ganglion cells. Donor vectors harboring homology arms to the target sequences designed to link the C-terminal region of the respective genes with fluorescent protein sequences are separated by a self cleaving hybrid spacer designed to reduce the likelihood of inadvertent cell function interference caused by misfolded or mislocalized fusion proteins. Pilot experiments have demonstrated that TALEN pairs designed to target the human AAVS1 locus can successfully modify HEK293 cells to constitutively express GFP in vitro.
Although much work remains in testing how well each of the respective constructs works in vitro, the complementary genome-editing tools adopted in this study have allowed us to generate an expansive collection of retinal cell-type specific reporter constructs that should greatly benefit investigations into both retinal development and disease modeling.

F-3162

EXOGENOUS FACTORS GOVERN THE PROPORTIONS OF PHOTORECEPTOR AND RETINAL PIGMENT EPITHELIAL PROGENITORS DERIVED FROM ADULT MOUSE RETINAL STEM CELLS

Brian G. Ballios, Saeed Khalili, Laura Clarke, Derek van der Kooy

Univ of Toronto, Toronto, ON, Canada

Adult retinal stem cells (RSCs) are rare pigmented cells in the ciliary epithelium (CE) of mice and humans. RSCs can give rise to retinal neurons including photoreceptors as well as retinal pigment epithelial (RPE) cells. We previously found that combinations of taurine, retinoic acid and FGF2/heparin (T+RA+FH) added to differentiating clonal RSC colonies increases the number of rods to 90% of all progeny. In contrast, 10% of RSC progeny produce rods when differentiated in 1%FBS+FH (pan-retinal conditions). We hypothesized that T/RA acts directly on RSC progeny in an instructive, rather than permissive, manner to bias photoreceptor differentiation, through the enrichment of rod-specific progenitors. We treated primary cultures of dissociated CE from adult mice with T+RA+FH or FH-only (normal RSC sphere media) for 7 d. When T+RA+FH-derived spheres were differentiated in 1%FBS+FH for 40 d, the percentage of cells expressing rhodopsin and RPE65 were increased and decreased, respectively, compared to FH-derived spheres differentiated in 1%FBS+FH. There were no differences in total cell number or the minor numbers of cells expressing Pax6 (retinal progenitors), calbindin (interneurons), or CRALBP (Müller glia). Differentiation of FH-derived spheres in BMP4 encourages RPE differentiation to over 80% of all progeny by 21 d (compared to 40% in 1%FBS+FH). When primary CE cultures were treated with BMP4 and then cultured in 1%FBS+FH for 28 d, there was no shift in the percentage of rod or RPE cells compared to FH-derived spheres. However, sphere growth in BMP4+T+RA+FH was sufficient to increase the proportion of rods. The higher number of rods arising from progenitors primed in T/RA, despite the presence of BMP4, suggests that T/RA is instructive for the production of NR progenitors during clonal RSC sphere formation. Since the proportion of RPE cells increased with higher BMP4 dose, with no change in the percentage of rods, BMP4 may be permissive for RPE progenitor differentiation. To test our hypothesis that T/RA is instructive for the production of lineage-restricted rod-specific progenitors we performed lineage analysis using limiting dilutions (on average <1 clone per well) of a fluorescent retroviral construct to label individual progenitor clones in vitro. Comparisons of the frequency and character of clones fated for rhodopsin-expression revealed an enrichment in the percentage of rod-only clones between 1%FBS (13%) to T/RA (over 70%), without affecting clone size or overall cell survival. A second approach made use of FACS sorting to isolate single non-pigmented and single pigmented cells in wells, which were then treated with T/RA for 28 d of differentiation. All surviving clones derived from non-pigmented progenitors (n=34) were rod-only clones (100% rhodopsin-positive), while those derived from pigmented progenitors (n=48) were predominantly no-rod clones (no rhodopsin-positive cells). Interestingly, one rod-only clone was found to be derived from a single pigmented cell (the largest of the pigmented cell-derived clones), suggesting potential neural lineage plasticity in very early pigmented progenitors. This study marks an important step towards the characterization of a rod-specific progenitor - no markers exist for such a cell, and the literature is divided on the existence of these cells in vivo. Furthermore, our study suggests a critical role for exogenous signals directing early lineage decisions between fate-restricted retinal progenitors.

F-3163

A BIOENGINEERED APPROACH FOR RETINA REGENERATION

Nikolaos Mitrousis[1], Roger Y. Tam[1], Derek van der Kooy[2], Molly S. Shoichet[3]
[A]Institute of Biomaterials and Biomedical Engineering, [B]Molecular Genetics, [C]Chemical Engineering and Applied Chemistry, [1]University of Toronto, Toronto, ON, Canada

Degenerative diseases of the retina are incurable and lead to blindness, with only pharmacologic therapy available to retard disease progression. Age-related macular degeneration (AMD) affects primarily the elderly, while retinitis pigmentosa affects children and working adults. A common aspect of the majority of these diseases is the death of the photoreceptors and retinal pigmented epithelium (RPE) of the retina. Stem cell regenerative therapy is a very promising approach to overcome blindness.
It has shown some potential in the eye, but there remain major obstacles to be addressed. Integration, survival and distribution of the cells are the main challenges. We have achieved improved cellular distribution after transplantation in the subretinal space of retinal stem cell progeny using an injectable delivery vehicle comprised of hyaluronan and methylcellulose (HAMC). The objective of the current study is to investigate the co-culture of human-derived RPE and photoreceptors in cell-adhesive HAMC hydrogels in vitro and ultimately in vivo. Towards this goal,

RPE were derived from hES cells (CA1 and H9 lines) following the overgrowth protocol established by Klimanskaya et. al with slight modifications. Rod photoreceptors were either derived by cell sorting from P4-P8 NrlGFP mice, or were differentiated from hES cells (H9 and H1 lines) using Dkk1, Noggin and IGF-1 (as described by Lamba et. al.). Cells were cultured in HAMC or HAMC modified with a fibronectin-mimicking peptide (RGD - HAMC-RGD). Both RPE and photoreceptors were efficiently derived from hES cells based on real time RT-PCR and immunostaining analysis. Culture in HAMC increased the viability of hES derived RPE cells, based on CalceinAM- ethidium homodimer staining. We found that the RPE cells express RHAMM and ICAM-1, two molecules known to mediate cell interaction with HA, but do not express CD44, another putative receptor for HA. When the two cell types were cultured in HAMC hydrogels, the percentage of cells remaining in the gel after 7 days was greater in HAMC-RGD (vs. HAMC alone). Additionally, while cells remaining in HAMC were round, those remaining in the HAMC-RGD gels formed processes, increasing the potential for interactions between RPE and photoreceptors. An HA-based tissue construct containing photoreceptors and RPE was achieved. This construct will be further evaluated in vitro and then in vivo for ultimate application in AMD or retinitis pigmentosa.

F-3164

SUSTAINED EFFECT OF BONE MARROW MONONUCLEAR CELL THERAPY IN AXONAL REGENERATION IN A MODEL OF OPTIC NERVE CRUSH

Camila Zaverucha-do-Valle, Louise Mesentier-Louro, Fernanda Gubert, Marcelo F. Santiago, Rosalia Mendez-Otero
Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

PURPOSE: In adult mammals, the regeneration of the optic nerve is very limited and at the moment there are several groups trying different approaches to increase retinal ganglion cell (RGC) survival and axonal outgrowth. One promising approach is cell therapy using bone marrow cells. In previous work, we performed intravitreal transplantation of syngeneic bone-marrow mononuclear cells (BMMCs) after optic nerve crush in adult rats and we demonstrated an increase in RGC survival and axon outgrowth 14 days after injury. In the present work, we investigated if these results could be sustained for a longer period of time.
METHODS: Optic nerve crush was performed in Lister-hooded adult rats and BMMC or saline injections were performed intravitreally shortly after injury. Neuronal survival and regeneration were evaluated in rats' retina and optic nerve after 28 days.
RESULTS: We demonstrated that there is an increase of 5.2 fold in the axon outgrowth 28 days after lesion, but the BMMCs effect on RGC survival was not sustained. In an attempt to prolong RGC survival, we established a new protocol with two BMMC injections, the first one soon after the lesion and the second one 7 days after the injury. Untreated animals received two injections of saline. We observed that although the axonal outgrowth was still increased after the second BMMC injection, the RGC survival was not prolonged.
CONCLUSIONS: These results demonstrate that BMMCs transplantation promotes neuroregeneration 28 days after injury. However, the effects on RGC survival previously observed by us at 14 days were not sustained at 28 days and could not be prolonged with a second dose of BMMC.

F-3165

COMPARISON OF TRANSPLANTATION OF MOUSE AND HUMAN RETINAL PROGENITOR: OBSTACLES THAT NEED TO BE OVERCOME

Caihui Jiang[1], Xin wang[1], Maonian zhang[1], Michael Young[2]
[1]Ophthalmology, Chinese PLA General Hospital, Beijing, China, [2]Ophthalmology, Schepens Eye Research Institute, Boston, MA, United States

Purpose: Many retinal degenerative diseases result in irreversible visual loss. Cell replacement therapy represents a novel approach for treatment of retinal degenerative diseases, such as retinitis pigmentosa and age-related macular degeneration. Work in the rodent has shown that retinal progenitor cells can exhibit markers representative

of mature photoreceptors following transplantation. Here we compare the results of transplantation of mouse and human retinal progenitor cells to learn the challenges in the use of human retinal progenitor cells.
Methods: Mouse and human retinal progenitor cells (RPCs) were isolated and expanded in vitro. Cells were characterized by immunochemistry for expression of retinal progenitor markers. Pre-labeled mouse and human RPCs in HBSS were transplanted into the subretinal space of mice. Mice were sacrificed 3 to 8 weeks after transplantation and eyes were collected for immunohistochemistry study.
RESULTS: Both mouse and human RPCs were able to survive after transplantation and migrate within the mouse retina and exhibit markers representative of mature photoreceptors. However, compared with mouse RPCs, much less human RPCs survived, migrated and integrated into the host retina and expressed markers representative of mature photoreceptors. In addition, the limited techniques of labeling human RPCs made it very difficult to study its morphology following transplantation.
Conclusions: There are challenges in the use of human RPCs to treat retinal degenerative diseases. Further research will be needed to overcome these obstacles.

F-3166

BONE MARROW-DERIVED STEM CELLS REGENERATE RETINAL NEURONS THROUGH A WNT-DEPENDENT CELL FUSION-MEDIATED REPROGRAMMING MECHANISM

Daniela Sanges, Neus Romo, Giacoma Simonte, Umberto Di Vicino, Maria Pia Cosma
Centre for Genomic Regulation, Barcelona, Spain

Reprogramming of a somatic nucleus back to a pluripotent phenotype can be induced in culture by cell fusion with high efficiency; however, whether this process occurs in vivo in mammalians remains enigmatic. Here, we show that spontaneous cell fusion can occurs between mouse retinal neurons and transplanted haematopoietic stem and progenitor cells (HSPCs) upon a retinal damage. Interestingly, activation of Wnt/βeta-catenin signalling induces the reprogramming in vivo of the retinal neuron genome within the fused hybrids; indeed, they reactivate the expression of neuronal and pluripotent precursor genes such as Oct4 and Nanog and can proliferate. Within the eye, the hybrids soon commit to differentiation towards a neuroectodermal lineage, and finally into terminally differentiated neurons, which can functionally regenerate the damaged retinal tissue. Following retinal damage and induction of Wnt signalling in the eye, cell-fusion-mediated reprogramming also occurs after endogenous recruitment of bone marrow-derived cells into the eyes. In conclusion, our data suggest that in-vivo reprogramming of terminally differentiated retinal neurons after their fusion with HSPCs can occurs in vivo and that this is a possible mechanism for neural tissue regeneration.

F-3167

RETROVIRAL TRANSDUCTION OF HUMAN RETINAL STEM CELLS USING ACOUSTIC WAVES

Yi-Ping Yang[1], Shih-Jen Chen[2], Hsin-I Ma[1]
[1]Department of Neurological Surgery, Tri-Service General Hospital and National Defense Medical Center, Taipei, Taiwan, [2]Department of Ophthalmology, Taipei Veterans General Hospital, Taipei, Taiwan

The pluripotent features of retinal stem cells (RSCs) on differentiating into neuron cells and photoreceptor cells endow RSCs with potential therapeutic properties for the field of regenerative medicine. In this study, we demonstrated the capability of RSCs developing into MAP2-expressing neuron cells and rhodopsin-expressing photoreceptor cells. To explore whether a specific gene can be passed along through lineage development, eGFP-encoded retroviruses were used to infect RSCs. The approach used here for retroviral transduction was acoustic wave fields created by ultrasound standing waves. Various operating parameters (e.g., exposure time and polybrene concentration) were investigated to determine the optimal conditions. Retroviral transduction efficiency to RSCs was augmented around 4 times higher than the one without exposure of acoustic waves in the presence of 8 μg/mL polybrene. With the concern of the acoustic wave effect on stem cell long-term performance, apoptotic activity of RSCs was conducted at different times of exposure as well as with various voltages applied to the system. Our results

indicate that significant cell apoptosis was detected under 25 Vp-p for acoustic exposure time exceeding 20 min. In conclusion, we demonstrated the suitable condition of ultrasound standing waves to achieve good efficacy for retroviral transduction into RSCs. We believe that this may be applied as experimental design in future studies and possible therapeutic use.

F-3168

SPATIALLY DIFFERENT INITIATION OF EMBRYOID BODY

Jiro Kawada[1], Shohei Kaneda[1], Hidenori Akutsu[2], Teruo Fujii[1]
[1]Institute of Industrial Science, The University of Tokyo, Tokyo, Japan, [2]National Research Institute for Child Health and Development, Tokyo, Japan

Formation of in vivo-like tissues in a dish is one of the major purposes in the stem cell research field. Recently it was proved that stem cells can self-assemble and form themselves into specific tissues under appropriate culture conditions. Previous studies have tried to reveal the process of stem cell differentiation and to apply it to the developmental biology and disease investigations. Now, it is imperative to develop a technique, which can analyze when and where a specific factor affects in a self-assembled heterogeneous tissue. However such analysis cannot be achieved in the conventional methods using dishes and multi-well plates.
Here, we propose a technique to induce spatially resolved differentiations of single embryoid body (EB) by soluble factors. To spatially expose specific differentiation factors, we use a culture insert, which has a polydimethylsiloxane (PDMS) membrane on the bottom. The bottom membrane has one pore of 200 μm in diameter, to immobilize an EB. First, we fill the well with a medium and put the insert into the well. Then an EB is injected into the insert with a culture medium different from the medium in the well. Once the surface level of the culture medium in the insert reaches higher than that of the one in the well, the flow is generated from the insert to the well through the pore by hydrostatic pressure. And the EB, which has a bigger diameter than the pore, will be carried by the flow and immobilized on the pore. Then, the two parts of the EB above and below the pore can be exposed to different factors.
We have cultured two mouse-ESCs (Rx-GFP K/I EB5 line provided by RIKEN Bioresource Center CELL BANK, Japan. This cell line expresses GFP in the retinal differentiated state,) -derived EBs together, one in the insert and the other in the well, with two different media. We made EBs at the cell concentration of 4000 cell/well with the retinal differentiation medium shown in Ref. [1]. We have put an insert into the well, in which an EB was already cultured, and another EB was injected into the insert to be immobilized on the pore. Then, we have injected the neural differentiation medium (ND medium) in the insert. By this technique, the two parts, above and below the pore of the EB can be exposed, respectively, to neural differentiation medium and retinal differentiation medium conditioned with another EB (RDC medium). Fluorescence images of the whole EB in the insert were taken by a confocal laser-scanning microscope shifting the focus with 10 μm step in height.
As a result, it was confirmed that only the part below the pore exposed to the RDC medium expressed GFP. And we could not observe GFP expression in the part above the pore. So we successfully could achieve spatially-controlled the retinal differentiation in a single EB.
This result is the first report of spatially-controlled differentiation of pluripotent stem cells into a specific tissue. This technique will lead to a break-through method to form and analyze differentiating types of tissues in vitro.
Reference
[1] M. Eiraku et al., “Self-organizing optic-cup morphogenesis in three-dimensional culture”, Nature, 472, 51, 2011

F-3171

TISSUE ENGINEERING OF THE HUMAN CORNEAL ENDOTHELIAL CELL (HCEC) LAYER

Aboulghassem Shahdadfar[1], Jesintha Navaratnam[1], Vinagolu K. Rajasekhar[2], Jon K. Slettedal[1], Agate Noer[1], Morten C. Moe[1], Liv Drolsum[1], Bjorn Nicolaissen[1]

[1]Department of Ophthalmology, Norwegian Eye Bank and Center for Eye Research, Oslo University Hospital and University of Oslo, Norway, [2]Memorial Sloan-Kettering Cancer Center, Stem Cell Center and Developmental Biology Program, Sloan-Kettering Institute, New York, New York 10065, USA, NY, United States

Purpose:
Cell loss from the human corneal endothelial layer in vivo is left uncompensated due to the non-proliferative status of the endothelium, and the final clinical result is corneal blindness. In the present study we investigate expression of selected markers associated with stemness and function in human corneal endothelial cells expanded ex vivo.
Methods:
Descemet's membrane with the attached endothelial cells was carefully dissected from human corneas in small strips. One part harvested as in vivo non-cultured cells and the other was cultivated in corneal endothelial cell growth medium for 6 weeks at 37^{o} C with 5% CO_2 in a humidified atmosphere and the medium was changed every 2-3 days. The pure cultivated HCEC population was seeded on acellular, non-synthetic carrier and cultivated for further 3 weeks in corneal endothelial cell expansion medium. Cultivated HCEC's on non-synthetic carrier and non-cultured HCEC (as control) were comparatively analyzed by qRT-PCR, electron microscopy (EM) and immunohistochemistry (IHM).
Results:
In our study the cultivated HCEC's seeded on non-synthetic carrier formed a stable monolayer similar to in vivo morphology of corneal endothelial cell layer. Our results show that the cultivated and seeded HCEC's on this biological carrier are functional and express stem cell and neural crest markers like in vivo HCEC's when analyzed by qRT-PCR, EM and IHM. The expression levels of markers associated with neural crest, stem cells and corneal endothelial cell functions *SNAI1, SNAI2, NES, SOX9, ZO-1, CX43, VADC2* and *VADC3* are significantly higher in tissue engineered HCECs compared to in vivo HCECs.
Conclusion:Endothelial loss is one major indication for lamellar transplantation using a donor cornea. Our preliminary results show that proliferation and expression of markers associated with stemness may be up-regulated ex vivo. Expansion of multiple grafts from selected donor corneas may to some extent compensate for the world-wide lack of donor corneal tissue.

F-3172

MICRORNA EXPRESSION PROFILES OF HUMAN IPS CELLS, RETINAL PIGMENT EPITHELIUM DERIVED FROM IPS, AND FETAL RETINAL PIGMENT EPITHELIUM

Whitney A. Greene[1], Alberto Muniz[1], Mark L. Plamper[2], Jae-Hyek Choi[1], Heuy-Ching Wang[2]
[1]Ocular Trauma, National Research Council, Washington, DC, United States, [2]Ocular Trauma, United States Army Institute of Surgical Research, San Antonio, TX, United States

Purpose: to compare the microRNA (miRNA) profiles of retinal pigment epithelium (RPE) derived from human induced-pluripotent stem (iPS) cells (iPS-RPE) to the miRNA profiles of undifferentiated human iPS cells and fetal RPE. Age-related macular degeneration (AMD) and retinitis pigmentosa (RP) are retinal diseases caused by degeneration of the RPE, a polarized layer of epithelium in the retina that performs multiple functions essential to the health of the neighboring photoreceptors. iPS-RPE is a potential source of cells that may be used to replace damaged RPE. To further characterize the iPS-RPE, we have compared the miRNA expression profile of RPE derived from iPS to the miRNA profiles of the parental iPS cells and fetal RPE. MiRNA previously shown to be expressed in RPE *in vivo* were selected for further analysis to determine the expression pattern during the differentiation process. MiRNAs with greater than 2 fold differential expression, either higher or lower, were selected for validation by RT-PCR and further analysis with Ingenuity IPA software to determine the miRNA target gene pathway.
Methods: iPS-RPE was derived from iPS cells. RNA was extracted from iPS, and fetal RPE and iPS-RPE cells after either 3 days or 17 days in culture, and then hybridized to the Agilent human v16 miRNA microarray. The miRNA expression profiles of iPS-RPE were compared to iPS cells to identify miRNAs that are either up or down regulated during the differentiation process. MiRNAs with greater than 2 fold differential expression, either higher or lower,

were selected for validation by RT-PCR and further analysis with Ingenuity IPA software to determine the miRNA target gene pathway. Finally, the miRNA expression profiles were cross-referenced with published data to identify miRNAs known to be expressed in ocular tissues and analyze their expression during the process of differentiation from iPS into RPE.
Results: Probes representing 1205 human and 144 viral miRNAs were used to analyze miRNA expression from each sample set. 94 miRNAs were differentially expressed in iPS cells compared to iPS-RPE day 3. 49 miRNAs were upregulated in the iPS-RPE compared to iPS, and 45 were downregulated in the iPS-RPE. 83 miRNAs that were differentially expressed in the iPS compared to iPS-RPE day 17, with 47 miRNAs upregulated in the iPS-RPE compared to iPS, and 36 downregulated in the iPS-RPE. 90 miRNAs were differentially expressed in fetal RPE compared to iPS-RPE day 3, with 57 miRNAs upregulated in the iPS-RPE, and 33 downregulated in the iPS-RPE. 110 miRNAs were differentially expressed in fetal RPE compared to iPS-RPE day 17, with 61 miRNAs upregulated in the iPS-RPE, and 49 downregulated in the iPS-RPE. Pathway analysis with Ingenuity IPA software indicated the differentially expressed miRNAs target transcripts for genes involved in cellular development, growth, proliferation and survival, cell cycle, and cellular movement. MiRNAs expressed in ocular tissues *in vivo* were found to be enriched in iPS-RPE and fetal RPE compared to iPS.
Conclusion: RPE derived from iPS are morphologically and functionally similar to fetal RPE. MiRNAs expressed in ocular tissues *in vivo* were also detected in iPS-RPE. The greatest number of differentially expressed miRNAS was detected in the fetal RPE versus iPS-RPE day 17 comparison, suggesting the iPS-RPE may be more mature at that timepoint. Future studies are planned to validate these results with RT-PCR.

F-3173

STEM CELL MODELING TO DEFINE THE MOLECULAR BASIS OF SCA7 ABSTRACT

Jacqueline M. Ward[1], Alysson R. Muotri[2], Albert R. La Spada[3]
[A]Biomedical Sciences Graduate Program, [B]Pediatrics, Cellular and Molecular Medicine, [C]Pediatrics, Cellular and Molecular Medicine, Institute of Genomic Medicine, [1]University of California, San Diego, La Jolla, CA, United States

Spinocerebellar ataxia type 7 (SCA7) is an autosomal dominant neurodegenerative disorder caused by a CAG/polyglutamine (polyQ) repeat expansion. It is characterized by cerebellar and retinal degeneration, which begins with a loss of color vision and central visual acuity, and ultimately progresses to complete blindness. Although SCA7 mouse models have provided key insights into mechanisms of disease pathogenesis, marked differences between mouse and human at the molecular, phenotypic, and therapeutic levels have revealed the need for a more representative model system to study this disease. We have generated an induced pluripotent stem cell (iPSC) model of SCA7 that can be differentiated to neural progenitor cells (NPCs) and retinal photoreceptors. We hope to use this photoreceptor model to fully characterize the molecular and cellular phenotypes to help better understand the retinal pathology. Phenotypes of interest include DNA damage, cell death, and chromatin decondensation. As ataxin-7 is a core component of the STAGA transcription co-activator complex, we also plan to use this model to study polyQ ataxin-7 induced transcriptional dysregulation through gene expression analysis and genome-wide location analysis to define alterations in ataxin-7 occupancy. Taken together, these studies will advance our understanding of the molecular basis of SCA7 retinal degeneration.

Neural Cells

F-3181

GASTRIN-RELEASING PEPTIDE IS AN ENDOGENOUS REGULATOR OF ADULT HIPPOCAMPAL NEUROGENESIS

Anoek de Koning, **Noah Walton**, Rick Shin, Katsunori Tajinda, Shinichi Miyake, Qian Chen, Adam Gross, Jeffrey Kogan, Carrie Heusner, Kouichi Tamura, Mitsuyuki Matsumoto
CNS, Astellas Research Institute of America, Skokie, IL, United States

Ongoing adult neurogenesis is a constitutive, dynamic process spanning the duration of life. While the majority of neurons in the brain are specified in utero, postnatal neurogenesis persists in the subventricular zone and dentate gyrus. Gastrin-releasing peptide (GRP) has multiple disparate roles throughout the body, including reported roles in the CNS (most notably in circadian rhythm). This ligand is one of three members of the bombesin pathway and acts via its cognate G protein-coupled receptor. Examination of GRP distribution in the brain reveals GRP expression occurs nearly exclusively in NeuN-positive neuronal cells. The exception to this are NeuN-negative, GRP-positive cells within the subgranular zone of the dentate gyrus, suggesting GRP may also play a role in neurogenesis and/or neuronal development. To investigate the relationship between neurogenesis and GRP expression, we compared a group of mice given free running wheel access for two weeks to sedentary controls. Running mice displayed higher levels of neurogenesis, lower levels of GRP-positive, BrdU-positive cells, and lower overall levels of GRP in the dentate gyrus, indicating that GRP expression has an inverse relationship with neurogenesis. To further investigate this relationship, we treated C57/Bl6 mice with GRP for ten days via intraventricular infusion. GRP treatment resulted in a decrease in proliferative cells, as well as an increase in phospho-CREB. Infusion of neuromedin B, another member of the bombesin family, did not affect neurogenesis or phospho-CREB expression. These results suggest that GRP may play a unique role in the maturation of proliferative progenitors to postmitotic neurons.

F-3182

GENETIC ANALYSIS OF NOTCH SIGNALING IN GLIOMA FORMATION

Claudio Giachino, Sebastian Lugert, Verdon Taylor
Department of Biomedicine, University of Basel, Basel, Switzerland

Gliomas are aggressive brain cancers with limited therapeutic options and poor prognosis for patients, particularly in late stage tumors. Genetic analysis has uncovered changes and mutations in a number of signaling pathways and intracellular pathway modulators. Although it remains controversial, neural stem and progenitor cells in the postnatal brain are believed to be one origin of gliomas. Notch signaling is required for neural stem cell maintenance, promoting quiescence and blocking differentiation. Accordingly, Notch signaling promotes a self-renewing stem cell-like state in glioma cells. However, whether glioma initiation from neural stem cells or progenitor cells and tumor progression depend on Notch signaling has not been demonstrated. We addressed the cell autonomous role of canonical Notch signaling in glioma cell biology using conditional genetics in multiple mouse models. We found that genetic deletion of RBPjk, the indispensable mediator of the canonical Notch signaling, does not impair platelet-derived growth factor-driven glioma growth in mice. However, RBPjk cooperates with known tumor suppressors to control cell proliferation, and loss of RBPjk and canonical Notch signaling promotes a premalignant state in neural stem cells. We addressed the interaction of Notch signaling with known glioma-associated genes and characterized the progression of Notch signaling-deficient cells towards transformation in vivo. We performed genome wide analysis of downstream targets of RBPJk in neural stem cells and glioma cells that may be associated with increased tumor formation. Our findings uncover fundamental differences in the molecular requirements of normal neural stem cells versus glioma cells and suggest that blocking Notch signaling may not always result in inhibition of glioma growth.

F-3183

DISC1 REGULATES SURVIVAL OF HUMAN NEURAL PROGENITOR CELLS VIA MITOCHONDRIAL MEDIATED APOPTOSIS: NEW INSIGHT TO HUMAN NEUROGENESIS RELEVANT TO NEURODEVELOPEMENTAL DISORDERS

Jeremy M. Crook[1], Shirani Sivarajah[1], Edwin K.H. Lim[2], Anita F. Quigley[3], Rob M. Kapsa[3], Mahesh Choolani[4], Efstratios Skafidas[1], Nao R. Kobayashi[1]

[1]Centre for Neural Engineering, The University of Melbourne, Parkville, Australia, [2]Stem Cell Disease Models, A-STAR Institute of Medical Biology, Singapore, Singapore, [3]Intelligent Polymer Research Institute, The University of Wollongong, Wollongong, Australia, [4]Obstetrics and Gynaecology, National University of Singapore, Singapore, Singapore

We have found a novel mechanism for the regulation of human neural progenitor cell survival that incorporates Disrupted in Schizophrenia-1 (DISC1) and mitochondrial-mediated apoptosis. Our findings are consistent with recent evidence that DISC1 plays a role in mammalian neurogenesis, and mediates this function via GSK3β/β-catenin signaling. Moreover, our results further explicate the adverse effects of DISC1 and GSK3β/β-catenin dysregulation on human brain development, which may be linked to apoptosis.

F-3184

A HIGH CONTENT SCREEN FOR COMPOUNDS THAT MODULATE NEURITE OUTGROWTH AND RETRACTION USING HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED NEURONS

Sean P. Sherman, Anne G. Bang

Conrad Prebys Center for Chemical Genomics, Sanford-Burnham Medical Research Institute, La Jolla, CA, United States

Pluripotent stem cells (PSCs), due to their capability to differentiate into any cell type in the body, have vast potential for providing in vitro models for studying human disease. This potential is particularly useful for the study of neurological disease where patient material would otherwise be impossible to obtain. Neurite formation plays a fundamental role in development and remodeling of neuronal networks under both normal and pathological conditions. This suggests that neurite outgrowth and retraction may be a useful phenotypic read-out to develop small-molecule probes to study neuronal network formation and how it is altered in neurological disease. We have developed a high content screening protocol for use in screening human induced pluripotent stem cell (hiPSC) derived neurons to identify small molecule regulators of neurite growth.

We demonstrate that hiPSC-derived neurons can be cultured on 384 well microtiter plates and are robust enough to withstand automated pipetting and washing techniques required for high throughput assays. Our initial screen uses immunostaining for the neural marker ßIII-tubulin (TUJ1) to identify neurites. Staurosporine, a broad kinase inhibitor, is used as a control compound, as we found that it can function as both an enhancer of neurite branching and an inhibitor of neurite outgrowth and cell health depending on the dose used. Our assay readouts include measures of neurite outgrowth and branching with Z-factors greater than 0.5, representing a robust screening assay. Cell health is assessed based on nuclear morphology. We will present results from screening a collection of 6000 bioactive compounds, primarily known drugs and well-characterized modulators of known targets to identify specific biological pathways and/or molecular targets that impact neurite outgrowth and retraction. This provides for the possibility to discover pathways and targets not previously known to play a role in these processes, as well as to discover tool compounds for further exploration of the underlying biology of neurite outgrowth and retraction.

This initial screen was developed using normal hiPSCs, however our screening platform will be further used to study disease patient iPSC-derived neurons in order to better understand changes in neuronal morphology, cell health, and neurite outgrowth associated with neurological disease.

F-3185

HEPARIN AND INDOMETHACIN ADMINISTERED AGAINST HOST RESPONSE MEDIATED NEURONAL INSULT DURING ACUTE BACTERIAL MENINGITIS INDUCE SELF REPAIR AND REGENERATION OF HIPPOCAMPAL CELLS IN WISTAR RATS

Daphne Santhosh[1], Indira Bairy[2]

[1]Cell and Molecular Biology, American University of Antigua College of Medicine, Coolidge, Antigua and Barbuda, [2]Microbiology, Melaka Manipal Medical College, MMMC, Manipal, India

Introduction and background:

Infectious meningitis caused by *Streptococcus pneumoniae* is associated with a high rate of mortality and morbidity in the geriatric patients and the very young. The fatality is the outcome of poor prognosis of the disease and may lead to severe neurological sequlae. About 30% of the survivors of pneumococcal meningitis develop long-term sequlae. The pathogenesis is due to the orchestration of pronflammatory cytokines like tumor necrosis factor-α (TNF-α), interleukin (IL)-1b and IL-6 and host immune response which are stimulated by the pneumolysin produced by *S.pneumoniae*. Even after the administration of antibiotics to treat and cure the disease, there is still the persistence of bacterial products and toxins still in the brain microenvironment.The coagulation pathway that is transduced simultaneously also contributes to brain injury associated with pneumococcal meningitis as they are instrumental in the formation of capillary thrombi leading to the death of neuronal cells.
The objective of the study was to investigate neuronal injury quantitatively in the sub regions of the hippocampus, after experimentally inducing meningitis in Wistar rats and assess the effect of indomethacin which is a non-steroidal anti-inflammatory drug (NSAID) that could curtail the process of proinflammatory damage mediated as a result of the host immune response to the pathogen and heparin that could negate the activity of the coagulation pathway and estimate the formation and birth of newly dividing cells in the hippocampus which gives a measure of self repair and regeneration during a neuronal insult caused by *Streptococcus pneumoniae* meningitis.

Materials and methods:

Meningitis was induced using oxacillin sensitive strain of *Streptococcus pneumoniae* ATCC 334400 suspended in Phosphate buffered saline (PBS) at $1x10^6$ CFU/ml and 10ul was incoculated into the cisterna magna of rats anaesthetized with 3%vol/vol halothane. Young Wistar rats (30 days old) were used for the study. The normal control group of rats were inoculated with 10µl saline on day 30. In the treated groups, meningitis induced rats were administered with indomethacin (1.2mg/kg body weight), heparin (4mg/kg body weight per 2ml) intraperitoneally. 0.5mg/kg body weight of Bromodeoxyuridine (BrDU) was injected in all the groups of animals intraperitoneally for four days. The animals were sacrificed on day 33. Bromodeoxyuridine (BrDU) staining was performed on 20-µm thaw-mounted cryosections spanning the entire hippocampus of both hemispheres.

Results and conclusions:

The results of the present experiment revealed that untreated pneumoccocal meningitis leads to extensive lesion in the sub regions of the hippocampus. In the rats that were treated with indomethacin, a considerable amount of self repair was observed than when compared to the ones treated with heparin. Although the adult hippocampus, a vital center for learning and memory, is extremely vulnerable to various insults such as ischemia, it is also known as an active neurogenic site, And in support of this, our investigation reveals, that during an insult like pneumococcal meningitis, there is active proliferation of newly dividing cells. And the significance of this study shows that addressing the inflammatory pathway damage through an NSAID like indomethacin, causes an increase in self repair and regeneration.

F-3186

RESVERATROL TREATMENT IN OLD AGE ENHANCES HIPPOCAMPAL STEM CELL ACTIVITY AND MAINTAINS NORMAL COGNITIVE FUNCTION

Ashok K. Shetty, Bharathi Hattiangady, Bing Shuai, Geetha A. Shetty, Xiolan Rao
Department of Molecular and Cellular Medicine, Institute for Regenerative Medicine, TAMHSC College of Medicine at Scott & White; Veterans Affairs Medical Center, Temple, TX, United States

Hippocampus, vital for learning and memory function, is one of the regions in the adult brain where neural stem cells (NSCs) maintain a unique form of plasticity called adult neurogenesis all through life. Because newly generated neurons from NSCs participate in hippocampal-dependent memory function, age-related decrease in hippocampal neurogenesis is believed to be one of the major causes of cognitive dysfunction in the elderly. Studies in aged rat models imply that aging does not reduce the numbers of putative NSCs but increases the percentage of quiescent NSCs in the hippocampus likely due to adverse changes in the NSC microenvironment. From this perspective, drugs capable of normalizing the altered NSC milieu in the aged hippocampus appear useful for stimulating NSCs to maintain higher levels of neurogenesis. Such drugs may preserve memory function in the normal aged population as well as promote functional recovery in elderly people afflicted with memory dysfunction. Resveratrol (RESV), a

phytoalexin found in the skin of red grapes with potent anti-inflammatory and antioxidant properties, strikes as an attractive drug for improving NSC activity and neurogenesis in the aged hippocampus because of the perceived link between reductions in NSC plasticity and increases in inflammation and oxidative stress with aging. Moreover, after the awareness of reduced cardiac risk in the consumers of RESV-containing red wine, RESV is being promoted as a dietary supplement. However, its effects on hippocampal neurogenesis and cognitive function in old age are unknown. We first examined whether oral administration of different doses of RESV (30, 60, 90 mg/Kg bw) for 28 days in aged (21-months old) F344 rats would enhance NSC activity and hippocampal neurogenesis. We measured net hippocampal neurogenesis via quantification of 5-bromodeoxyuridine (BrdU) positive newly born cells and analyses of BrdU+ cells expressing NeuN in the subgranular zone-granule cell layer of the hippocampus, at 38 days after the termination of twelve daily BrdU injections performed on RESV treatment days 17-28. We found increased net hippocampal neurogenesis in aged animals treated with RESV at 60 mg/Kg bw and 90 mg/Kg bw. Analyses of the status of hippocampal neurogenesis at 38 days after the conclusion of RESV treatment via counting of doublecortin (DCX) positive newly born neurons also revealed a similar trend. We next examined whether RESV treatment would maintain normal memory function with advanced age. We treated two cohorts of aged (19 months old) rats exhibiting comparable learning and memory function in a water maze test with RESV (90mg/Kg/day) or vehicle (VEH) for 28 days. Two months later, both groups of rats were tested for reference and working memory function through additional water maze tests. Aged rats receiving VEH showed both reference and working memory dysfunction whereas aged rats receiving RESV exhibited normal memory function. Additional analyses revealed reduced concentration of pro-inflammatory cytokines (TNFα and IL-1β) in the hippocampus of aged rats receiving RESV but not in aged rats receiving VEH. Collectively, these results suggest that RESV treatment in old age is beneficial for enhancing hippocampal neurogenesis and maintaining normal memory function. Modulation of NSC milieu in the aged hippocampus through reductions in the concentration of pro-inflammatory cytokines may underlie RESV-mediated functional benefits. Supported by a grant from NIH-NCCAM (AT006256).

F-3187

USING MICE **WITH HUMAN IMMUNE SYSTEMS TO MODEL HUMAN NEURAL STEM CELL TRANSPLANT ACCEPTANCE**

Juan Carlos Biancotti, Joshua J. Breunig, David Gate, Javier Rodriguez, Clive N. Svendsen, Terrence C. Town
Cedars-Sinai Medical Center, Los Angeles, CA, United States

Category: Immunology and Stem Cells
Secondary Category: Neural Cells
Using mice with human immune systems to model human neural stem cell transplant acceptance

Juan Carlos Biancotti[1], Joshua Breunig[1], David Gate[1], Javier, Rodriguez Jr.[1], Clive Svendsen[1,2], and Terrence Town[1,2]

[1]Regenerative Medicine Institute and Department of Biomedical Sciences, Cedars Sinai Medical Center, Los Angeles, CA 90048

[2]Department of Medicine, David Geffen School of Medicine at the University of California, Los Angeles, Los Angeles, CA 90048

Abstract:A key issue in stem cell transplant biology is solving the problem of transplant rejection. Despite extensive research in human embryonic stem cells (hESC), little is known about the factors governing immune system tolerance to hESC-derived grafts. Our work directly addresses this important issue by investigating immune system components known as human leukocyte antigens (HLA). Because mouse and human immune systems fundamentally differ, we have established cutting-edge mouse models that have human immune systems. Such models rely on immunocompromised mice as recipients for human umbilical cord blood-derived stem cells. These mice go on to develop a human immune system, complete with HLAs, and have been used to engraft hESC-derived neural progenitors that are either HLA matched or mismatched. Here, we show successful establishment of mice bearing > 50% reconstitution with human immune cells, including T and B lymphocytes that form the basis for adaptive immune responses that are central cellular players in graft acceptance vs. rejection. Additionally, we have successfully HLA genotyped hESC lines and determined the expression pattern of HLA subtypes. Neural progenitors derived from

these cell lines have been transplanted into brains of recipient humanized mice. Immunohistochemical analysis of these brains has demonstrated immune reaction against neural progenitors with mismatched HLA haplotypes. The use of these next-generation humanized mouse models is expected to allow us to determine the role(s) of HLAs in human neural stem cell transplant tolerance.

F-3188

CELL-SPECIFIC FRATAXIN DEFICIENCY IN PERIPHERAL SENSORY NEURONS IN A FRIEDREICH ATAXIA MODEL BASED ON HUMAN INDUCED PLURIPOTENT STEM CELLS

Andreas Eigentler[1], Sylvia Boesch[1], Georg Dechant[2], Roxana Nat[2]
[A]Department of Neurology, [B]Institute for Neuroscience, [1]Innsbruck Medical University, Innsbruck, Austria

Background and objective: Friedreich Ataxia (FRDA), an autosomal recessive neurodegenerative disease with an intronic GAA repeat in the gene encoding the mitochondrial protein frataxin (FXN), is one of the most common forms of hereditary ataxia. Proprioceptive sensory neurons of the dorsal root ganglia are particularly susceptible for neurodegeneration in FRDA patients. With the aim to establish a new in vitro model of the disease, we describe here a differentiation protocol for human induced pluripotent stem cells (iPSC) towards neural crest cells and peripheral sensory neurons.
Methods: IPSCs were generated via transduction of primary dermal fibroblasts with a polycistronic lentiviral vector. Furthermore, we applied a directed differentiation protocol modulating the canonical Wnt/β-catenin signaling cascade in order to generate neural crest progenitors and eventually peripheral sensory neurons. Immunocytochemistry, qRT-PCR, as well as genetic profiling and Western blot analyses were performed to thoroughly characterize each of the obtained cell populations.
Results: We generated and validated iPSC lines derived from two patients with clinically and genetically confirmed FRDA, and a disease duration of about 15 years and from two healthy individuals. Thorough analyses proved the pluripotent nature of the obtained cell lines. The central FRDA pathologic hallmarks, the GAA triplet repeat expansion as well as the subsequent reduction of FXN level, were conserved in FRDA-iPSCs and their derivatives. We detected a disproportionally strong deficiency of FXN levels in the early FRDA peripheral sensory neuronal populations.
Conclusion: The analysis of FXN expression dynamics during neuronal in vitro development constitutes a solid step forward in FRDA disease modeling. Our results emphasize the correlation between reduced FXN expression levels and disease susceptibility in FRDA and further our understanding of how FXN expression connects with cell-specific neurodegeneration.

F-3191

DECONVOLVING HUMAN CORTICOGENESIS BY GENE COEXPRESSION ANALYSIS

Chandrasekhar S. Raju, Michael C. Oldham
Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California San Francisco, San Francisco, CA, United States

The human neocortex is an extraordinarily complex, heterogeneous structure comprised of many different cell types and greatly enlarged compared to the neocortices of other mammalian species. Understanding how the human neocortex normally develops in utero holds the key to understanding our vulnerability to malformations of cortical circuitry, which are believed to contribute to the autism spectrum and many psychiatric disorders. Several groups have tried to characterize the molecular and cellular composition of the developing neocortex by analyzing gene expression differences among subpopulations of cells isolated using laser-capture microdissection or other techniques. Our objective in this study was to test an alternative approach for deconvolving the cellular composition of the developing human neocortex by analyzing patterns of gene activity. Here, we report that we have developed an unbiased method called Gene Coexpression Analysis of Serial Sections (GCASS) that can identify molecular signatures of distinct cell types using only a single tissue sample. Using this approach, we have generated

two microarray data sets, each consisting of 96 samples sectioned in series from the developing cortex of one human individual (18 and 22 gestational weeks). By systematically analyzing gene coexpression relationships in these datasets, we have obtained molecular signatures of radial glia (the neural stem cells of the developing cortex), microglia, ependymal cells, nascent excitatory neurons, and migrating GABAergic neurons, among others. Our results provide an integrated functional context for studying genes that have been implicated in neurodevelopmental disorders, while simultaneously identifying novel regulatory factors and biomarkers associated with the production and maturation of myriad human cortical cell types.

F-3193

BDNF DYSREGULATION IN HUNTINGTON'S DISEASE: SEEKING NEW INSIGHTS USING NEURAL DERIVATIVES OF DISEASE-SPECIFIC HUMAN PLURIPOTENT STEM CELLS

Fany BOURGOIS-ROCHA[1], Sophie AUBERT[2], Diana ZALA[3], Valérie DROUET[4], Marta RUIZ[4], Jérémie CHARBORD[1], Caroline BONNEFOND[2], Benjamin BRINON[2], Marc PESCHANSKI[1], Philippe HANTRAYE[4], Frédéric SAUDOU[3], Nicole DEGLON[5], Anselme Louis PERRIER[1]

[1]Inserm/UEVE UMR 861, I-Stem, AFM, Evry, France, [2]CECS, I-Stem, AFM, Evry, France, [3]Institut Curie/CNRS UMR146, Orsay, France, [4]CEA/ I2BM/ MIRCen, CNRS URA2210, Fontenay-aux-Roses, France, [5]CHUV/LNCM, CEA/ I2BM/ MIRCen, CNRS URA2210, Lausanne, Switzerland

Huntington's disease (HD) is an autosomal dominant disorder characterized by motor, cognitive, psychiatric and behavioral disturbances. This devastating neurodegenerative disease is caused by the expansion of CAG repeats in the Huntingtin gene (*HTT*). HTT protein is involved in multiple cellular functions, many of which are impaired in HD cells, such as for example transcriptional regulation and intracellular transport. In HD cortical neurons, Brain-Derived Neurotrophic Factor (BDNF) regulation is disrupted both at the transcriptional level and at the level of its vesicular axonal transport. BDNF is produced by cortical neurons and required for survival and differentiation of striatal neurons, which are the type of neurons primarily affected in HD. This highlights the importance of a better understanding of BDNF disruption in human neurons carrying the most common forms of the HD mutations (i.e. CAG expansions in the range of 40 to 60).
We investigated the impact of such HD mutations on BDNF protein vesicles transport in neurons generated from HD human embryonic stem cell (HD-hESC) lines derived from pre-implantation genetically diagnosed embryos. We found that the defect demonstrated in other HD genetic models was recapitulated in these human neurons. To explore in more details the role of normal or mutant HTT in this process we knocked down specifically one of the two HTT allele using allele-specific shRNA vectors. These vectors target heterozygous single nucleotide polymorphisms (SNP) previously associated with one of the two *HTT* alleles. Allele specific knock-down of mutant HTT rescued BDNF transport in HD neurons while knock-down of WT-HTT had no impact, arguing in favor of a dominant negative effect of mutant HTT protein on axonal transport.
Currently, we are exploring the role of HD mutations on BDNF transcriptional regulation focusing on the transcriptional repressor REST. This protein regulates transcription by binding on its target gene promoters via a consensus nucleotide sequence name RE1. RE1 sequences are found in the promoter of many neuronal genes, including at least one of the BDNF promoters in the mouse and human genome. HD mutations have previously been shown, in other models, to lead to abnormally high REST activity, which in turn induces a reduction in BDNF expression level. Study of the role of normal or mutant HTT in this dysregulation, in HD and WT neural stem cells and neurons derived from hESCs, using the allele-specific knock-down strategy, a luciferase-reporter assay and QRT-PCR is ongoing.

F-3194

GABAERGIC DIFFERENTIATION AND BEHAVIORAL IMPROVEMENT BY BDNF-OVEREXPRESSING HUMAN NEURAL STEM CELLS (HB1.F3.BDNF) IN A RODENT MODEL OF HUNTINGTON'S DISEASE

Iksoo Jeon[1], Nayeon Lee[1], Da-Jeong Chang[1], Chunggab Choi[1], Hyun Sook Kim[1], Seung-Hun Oh[1], Young Seok Park[1], Jihye Kwon[1], Hyunseung Lee[2], Kwan Soo Hong[2], Hong J. Lee[3], Seung U. Kim[3], **Jihwan Song**[1]
[1]CHA Stem Cell Institute, Department of Biomedical Science, CHA University, Seoul, Korea, Republic of, [2]Division of Magnetic Resonance, Korea Basic Science Institute, Ochang, Korea, Republic of, [3]Medical Research Institute, Chung-Ang University College of Medicine, Seoul, Korea, Republic of

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder that is caused by abnormal expansion of CAG repeats in the *huntintin* gene. The huntingtin mutation leads to the progressive and selective degeneration of striatal GABAergic medium spiny projection neuron. Brain-derived neurotrophic factor (BDNF) is known to be reduced in HD patients' brain and is required for the correct activity of the corticostriatal synapse and the survival of the GABAergic neurons. In this study, we transplanted human neural stem cells overexpressing BDNF (HB1.F3.BDNF) into the contra-lateral side of striatum at 7 days after quinolinic acid (QA) was injected to the rat brains unilaterally, and the transplanted animals were monitored up to 6 weeks using various behavioral tests. Interestingly, we observed that the rats receiving HB1.F3.BDNF cells exhibited significant behavioral improvements in stepping, rotarod and apomorphine tests. To track the fates of transplanted cells in vivo, we employed 4.7T animal MRI, which visualized the migration of contra-laterally transplanted cells to the lesion site, as well as the reduction of lateral ventricle. Histological analyses further revealed that the transplanted cells were differentiated into GABAergic, MSN-type neurons, judged by the expression of DARPP-32, GABA and GAD65/67-positive cells. Using a retrograde tracer (Fluoro-Gold), we demonstrate that the migrated transplanted HB1.F3.BDNF cells to the lesion site were functionally connected to the neuronal network of the host brains. Finally, we observed that the transplanted animals exhibited a significant reduction of inflammatory response (ED1), compared with sham controls. Taken together, these results indicate human neural stem cells overexpressing BDNF (HB1.F3.BDNF) are effective in treating HD.
This study was supported by a grant of the Basic Science Research Program through the National Research Foundation of Korea (NRF)funded by the Ministry of Education, Science and Technology (2012R1A1A2006827), Republic of Korea. We are grateful to the MRI
facility in the Division of Magnetic Resonance, Korea Basic Science Institute, Ochang, Korea.

F-3195

3D CULTURES FOR HUMAN PLURIPOTENT STEM CELL -DERIVED NEURONAL CELLS

Marja Pajunen, Tiina Joki, Laura Ylä-Outinen, Mari Varjola, Heli Skottman, Susanna Narkilahti
Institute of Biomedical Technology, BioMediTech, University of Tampere, Tampere, Finland

Background. Neurodegenerative disorders like Parkinson's and Alzheimer's diseases are associated with permanent damages to cells and brain structures. Encouragingly, there have been some success with neural transplants, however, the cell survival after grafting is still a major challenge. To improve the survival, the cells should be incorporated into protective biomaterials. These biomaterials should be non-toxic, three dimensional, support the cell viability and allow nutrition flux. In addition to the protection of the cells, biomaterials offer a more natural, tissue-like environment for the cells to grow in. For neural applications hydrogels are considered to be the best option. Here, we have tested the feasibility of the commercially available hydrogel PuraMatrixTM as an in vivo growth platform for human pluripotent stem cell -derived neural cells.
Methods. Human pluripotent stem cell -derived neural cells were seeded to different hydrogel concentrations on top of the gelled material, inside the hydrogel, or under the hydrogel. The cells were characterized using viability analysis, immunocytochemical staining, time-lapse monitoring, and confocal imaging. In addition, the maturation and electrical activity of the neuronal networks inside the hydrogel were characterized using microelectrode array.
Results. The hydrogel was non-cytotoxic and supported the survival of neural cells, both neurons and glial cells. Moreover, neurons inside the gel developed more branched neurite structures compared to the traditional 2D culture. Importantly, these neurons were also able to form spontaneously functional networks.

Conclusions. Even though there are some challenges related to this material, like the drastic pH changes during gelation, our data encourages to further study this material for a growth matrix for human-derived neural cells. Also, further studies of neuronal functionality in 3D matrices are conducted.

F-3196

A CHEMICAL APPROACH TO GENERATING INTEGRATION-FREE INDUCED NEURAL PROGENITOR CELLS

Mingliang Zhang, Sheng Ding
The J David Gladstone Institute, San Francisco, CA, United States

Recent studies with iPSC factors established a paradigm of employing epigenetic activation with transient iPSC factor expression in fibroblast cells in conjunction with modulating defined developmental signaling pathways by growth factors to reprogram somatic cells from one cell type into another, conventionally known as transdifferentiation. Although this approach has been demonstrated to generate induced cardiac, neural, endoderm and endothelial cells from both mouse and human fibroblasts, its efficiency is quite low and it typically involves gene integration. To improve the efficiency of this process and the quality of the resultant induced neural stem cells (iNSCs), we used an episomal expression system of OSKM, and performed a high-content phenotypic chemical screen of synthetic compounds. A small molecules cocktail that could promote generation of nestin/pax6 double positive neural stem cells from mouse embryonic fibroblast was eventually identified. Cells with the treatment of cocktail show more than 20 folds increase of the generation of iNSCs. The resulting integration-free iNSCs exhibit uniformly cell morphology and molecular features resemble the primary neural stem cells. The iNSCs possess tripotency to differentiate to neuron, astrocyte, and oligodendrocyte in vitro. These iNSCs could be expanded for long-term in vitro without loss tripotency. Gene expression analysis indicates that (1), the pro-neural genes required for neural fate determination, such as Shh, Ntf3, Hes5, and Nkx2-2, were greatly up-regulated at the early stage;(2), the neural fate determine genes, such as Ascl1, Pax6, and Sox1, are also increased; and (3), the neuronal fate induction genes, such as NeuonD1, NeuroG2, are increased and the repressive gene REST is down-regulated. These results highlight a neural induction function of the cocktail. Practically, this small molecule cocktail makes the non-specific cell fate conversion process induced by OSKM to a specific process to neural induction process. This approach could be useful to obtain lineage specific stem cells with higher efficiency and quality, and hold the promise to drug discovery for neurodegenerative disease. The mechanistic study could broaden our knowledge of the molecule events related to cell fate conversion, especially at the early stage of OSKM-induced reprogramming/transdifferentiation.

F-3197

DIRECT REPROGRAMMING OF HUMAN FIBROBLASTS TO FUNCTIONAL NEURONS WITH SELF-REGULATING NON-INTEGRATIVE VECTORS

Shong Lau[1], Johan Jakobsson[2], Malin Parmar[1]
[A]Developmental Neurobiology, [B]Molecular Neurogenetics, [1]Lund University, Lund, Sweden

Direct conversion of human fibroblasts into functional neurons, induced neurons (iNs), has great potential in the treatment of neurological disorder with cell transplantation. Recent measures of generating iNs relies on integrating viral vectors and a continuous expression of the reprogramming factors, which hampers their clinical translation. Our study is aimed at generating human iN cells with reprogramming factors (Ascl1, Brn2 and Myt1l) that are regulated by microRNA. The microRNA regulation is achieved by lentiviral vectors that encode reprogramming factors followed by microRNA target (mirT) sequences of an endogenous neural lineage specific miRNA, mir-124. This allows expression in fibroblasts and degradation of the transgene specifically in cells that have converted into neurons. We were able to show that converted iN cells express mir-124. To examine the regulatory potential of the microRNA on the target transgene, we utilized a GFP reporter construct with mirT sequence. Converted iN cells were stained for NCAM and among these NCAM positive cells we observed a 50% GFP down-regulation in the regulated construct. On the other hand, we were able to generate human iN cells expressing MAP2 and NCAM with reprogramming factors (Ascl1, Brn2 and Myt1l) delivered by integration deficient lentiviral vectors and the mirT

regulated vectors generate human iN cells at a similar efficiency as the non-regulated ones. This study provides evidence that human fibroblasts can be directly converted into neurons with non-integrating, self-regulating transgenes.

F-3198

INFLUENCE OF AGE RELATED GENES ON NEURONAL CONVERSION FROM AGING FIBROBLASTS

Henrik Ahlenius[1], Soham Chanda[1], Ashley E. Webb[2], Anne Brunet[2], Thomas C. Südhof[3], Marius Wernig[1]
[1]Institute for stem cell biology and regenerative medicine, Stanford, CA, United States, [2]Department of Genetics, Stanford, CA, United States, [3]Molecular and Cellular Physiology, Stanford, CA, United States

Defined sets of transcription factors have recently been used to convert mouse and human fibroblasts into cells with neuronal characteristics. These induced neuronal (iN) cells show morphological, biochemical and functional hallmarks of mature neurons. So far most studies have focused on embryonic fibroblasts. However, for many applications it would be preferable to generate iN cells from adult and aging cells. Aging is associated with declined cognitive function, changes in neural plasticity and reduced neurogenesis. On the other hand it is difficult to study intrinsic neuronal properties of aged mammals. It would therefore be desirable to generate aged iN cells in order to study age related changes in neurons.
Here we investigated if iN cells can be generated from aged cells, whether there are any changes in reprogramming efficiency, function of aging iN cells and if these potential differences can be modulated by expression of age related genes.
We generated iN cells from embryonic to adult up to 25 month old, mouse fibroblasts. Fibroblast cultures were established from different aged mice and infected with doxycycline-inducible lentiviral vectors carrying Brn2, Ascl1 and Myt1l. iN cell generation and function was assessed at 1-4 weeks after transgene induction. iN cells from all ages displayed mature neuronal morphologies and were positive for the pan-neuronal markers Tau, Tuj1 and Map2. There was a significant drop in the efficiency of iN cell conversion in embryonic to postnatal fibroblasts and a further decrease from postnatal to adult fibroblasts. Furthermore, embryonic and postnatal fibroblast converted faster as compared to adult and aged fibroblasts. However, we could not detect any change in iN cell conversion efficiency from fibroblasts aged 3 to 25 month.
To assess the functional properties of aging iN cells, we next performed electrophysiological experiments. Upon current injection, iN cells from different ages generated single or multiple action potentials. Furthermore, when co-cultured on primary hippocampal cultures iN cells from different ages formed synapses and exhibited spontaneous and evoked postsynaptic currents. The efficiency of forming functional iN cells was higher in embryonic compared to postnatal, adult and aged fibroblasts.
In an attempt to increase the efficiency of iN cell conversion from aged fibroblasts we are now systematically investigating the effect of age-related genes and pathways such as Sirtuins, NFk-B pathways and FoxO family of transcription factors on the reprogramming of fibroblasts to iN cells. In summary, we here show that functional iN cells can be generated from aged mouse fibroblasts, a process that is regulated by age-related gene expression and suggest that these findings may be useful for studying neuronal aging and to model age related changes in neurogenesis.

F-3201

DETECTION OF MICRORNA HETEROGENEITY IN SINGLE CELLS USING AN AUTOMATED MICROFLUIDIC CHIP SYSTEM

Anne Leyrat, Joseph Shuga, Nianzhen Li, Lukasz Szpankowski, Marc Unger, Jay A. West
Research and Development, Fluidigm, South San Francisco, CA, United States

Detection of MicroRNA heterogeneity in single cells using an automated microfluidic chip system
A. Leyrat, J. Shuga, N. Li, L. Szpankowski, M. Unger1 & J. A. West1,;
1New Technologies, Fluidigm Corporation, South San Francisco, CA

The expression of small or micro RNA (miRNAs) species in cellular populations is thought to drive downstream gene expression and protein functionality. miRNAs are short (18–24 nucleotides), non-coding RNAs that regulate gene expression by both disrupting messenger RNA (mRNA) stability and inhibiting their translation. Our goal was to determine the variability in miRNA species at the single cell level using a microfluidic system which automates single cell capture
and miRNA pre-amplification for downstream expression analysis. We have developed a simple, modular workflow for streamlined analysis of cell populations down to the single-cell level. The workflow is centered on two key components: cell isolation and cDNA preparation and highly parallel gene expression analysis using a dynamic array device. Using this platform cDNA samples are then loaded and analyzed in parallel with up to 96 gene expression assays in parallel on Dynamic Array™ IFCs using the Biomark HD system. We demonstrate the ability to detect significant variation in the expression of discrete miRNA species in a population of single cells from a single phenotype. Comparison of phenotypically distinct populations (human embryonic fibroblasts, human induced Pluripotent Stem Cells and fully differentiated human neurons), demonstrate more dramatic differences in addition to the heterogeneity of expression within each group.

F-3202

INTERACTIONS OF MOUSE EMBRYONIC STEM CELL-DERIVED NEURAL PROGENITORS WITHIN THE HOST BRAIN

Chelsea Lassiter, Sandy Becker, Laura Grabel
Wesleyan University, Middletown, CT, United States

Embryonic stem cell (ESC)-derived transplants hold great promise as cell replacement therapies for neurodegenerative diseases, and it is important to understand the host brain conditions that will lead to a successful transplant. We use a fluid lesion model, transplanting mouse or human ESC-derived neural progenitors (ESNPs) into the hippocampi of mice that have been given a kainic acid-induced acute seizure. Within a week after transplantation, the ESNPs have migrated posteriorly along the upper blade of the dentate gyrus (DG), replacing the endogenous cells of the upper blade that have degenerated due to the fluid injection. After four weeks the transplanted ESNPs have integrated into the upper blade, and our preliminary data suggest that the transplant recruits host endothelial cells from the surrounding hippocampus to form vessels. We also observe that migrating ESNPs are found in close proximity to endogenous blood vessels. Our previous data suggest that the posterior migration of the transplanted cells occurs in response to the chemokine CXCL12 (SDF-1α), and we now show that this chemokine is present on the host vasculature and astrocytes, with the receptor for CXCL12, CXCR4 present on our transplanted cells. Our data so far suggest a role for blood vessels in supporting the transplant and in providing a migratory scaffold similarly observed within the subventricular zone (SVZ). Our data suggests that neural progenitors (NPs) migrate along vessels within the hippocampus after transplant and other labs have shown this to occur in other areas of the brain, both with endogenous and transplanted cells. Astrocytes have been shown to regulate vascular development in the SVZ, which aids in the correct migration of neuroblasts during development. In our model, we propose that astrocytes are responsible for signaling to blood vessels through CXCL12 within the hippocampus, which allows for successful migration of our transplanted ESNPs. We also use hippocampal brain slice culture as a method for studying this migration and the signaling responses that may be occurring. Preliminary data suggests that when our ESNPs are seeded onto hippocampal slices, they associate with the blood vessels during culture. We aim to identify the molecular factors that regulate this behavior to aid in producing a more successful transplant.

F-3203

GLOBAL PROFILING OF MIRNA EXPRESSION INDENTIFIES POTENTIAL NEW MIRNAS INVOLVED IN DORSAL FOREBRAIN NEURAL PROGENITOR INDUCTION

Sherman Ku, Yanling Wang, Vilas Menon, Angel Nelson, Refugio Martinez, Ajamete Kaykas, Shuyuan Yao
Allen Institute for Brain Science, Seattle, WA, United States

MicroRNAs (miRNAs) are involved in many regulatory pathways. Profiling of miRNA provides a new approach to identify molecular mechanisms that control biological processes. Using a modified version of a previously developed method for neural induction of human embryonic stem cells (ESCs) via dual-SMAD inhibition, we differentiated H1 ESCs to early PAX6+/FOXG1+ dorsal forebrain progenitors at high yield (>95% by flow cytometry) over the course of twelve days. Total RNA was collected every three days during this induction period from three independent experiments, and samples were submitted for global miRNA expression profiling (Sanger miRBase version 18). Global expression data were filtered for expression level thresholds, and statistically significant differential expression was calculated by ANOVA. Forty-two miRNAs were identified based on a Bonferroni-corrected significance cutoff of $p < 0.05$, twenty-four of which were highly up-regulated during induction and eighteen of which were highly down-regulated during induction. Presumably, the eighteen members which were highly down-regulated are associated with pluripotency. Indeed, previously reported miRNAs such as miR-367 and the miR-302 cluster were identified. However, others identified by this data have not been described to be associated with pluripotency in the literature. Similarly, many of the down-regulated miRNAs are undescribed in the literature as well. These data suggest that there are novel miRNAs that can be used to report on this differentiation process or, more strikingly, that there are miRNAs that perhaps regulate this process in a novel manner.

F-3204

HUMAN NEURAL STEM CELLS IN A MODEL OF INDIRECT DEVELOPMENTAL NEUROTOXICITY CAUSED BY METAL NANOPARTICLES.

Simon J. Hawkins, Lucy Crompton, Aman Sood, Federica Rinaldi, Patrick Case, Maeve Caldwell
School of Clinical Science, University of Bristol, Bristol, United Kingdom

Human neural stem cells are increasingly being used to model neurological disease. In this study we have utilized fetal cortical tissue as a renewable source of neural stem cells (NSCs) to investigate the potential for metal nanoparticles (NPs) to cause developmental neurotoxicity across a cellular barrier. This barrier is created by growing bewo choriocarcinoma cells on polyester inserts for 7 days that contain 0.4µm pores with media in chambers above and below the insert. By 7 days the cells have formed a confluent bilayered barrier that is then exposed to CoCr NPs in the upper chamber for 24 hours followed by collection of media in the lower chamber below the barrier. This model has been extensively tested previously demonstrating that the NPs in this setup do not cross the barrier and are instead internalized and initiate a signaling cascade within the barrier.
NSCs were dissociated and allowed to differentiate in NSC media containing B27 and minus growth factors for 7 days after which the cells were collected for analysis. On the second day the NSC media was exchanged for NSC media from the barrier model or standard NSC media. A comparison was made between the effects of transferring media onto the NSCs from the bewo model exposed to metal NPs (BM) compared to control samples of standard media (C) and media from the barrier model not exposed to NPs (B).
Toxic effects on the neural cells were assessed using a Live Dead cell assay that found no difference. Cell fate was then assessed using glial fibrillary acidic protein immunostaining to identify astrocytes and beta tubulin immunostaining to identify neurons. Media transferred from the barrier setup with or without NP exposure (B and BM) was found to result in a 14% increase in astrocytes within the culture when compared to standard media control (C). Interestingly, however, the exposed astrocytes (BM) showed marked alteration in their morphology within the resulting mixed culture when compared to controls (C and B). The astrocytes nuclear and cytoplasmic area had increased but without alteration in the nuclear cytoplasmic ratio. It was hypothesized that this could be due to DNA damage so immunostaining for gamma H2AX foci, a marker of double stranded DNA breaks, was performed. This showed a seven fold increase in gamma foci in the GFAP stained astrocytes and a small increase in beta tubulin positive neurones in the mixed culture likely reflecting diminished cellular support from DNA damaged astrocytes.
As signalling molecules passing through connexin 43 (Cx43) gap junctions or hemi channels have been previously implicated in signalling induced through the barrier; experiments were performed to investigate this in our model. GAP 26 connexin mimetic peptide, a selective blocker of Cx43 hemichannels and gap junctions, was added at the same time as the CoCr NPs exposure in the barrier model (BM+GAP). Provisional results have showed that this decreases the formation of gamma foci in the astrocytes in mixed culture. Further exploration of the role of Cx43

channels is currently being made using viral ShRNA mediated knockdown and overexpression of Cx43 in the NSCs and in the bewo barrier cells.
We conclude that we have successfully used human NSCs to model indirect NP induced neurotoxicity. Developing astrocytes are particularly affected by this insult and neurones are secondarily affected. Finally we believe that connexin 43 channels within our barrier model may be involved in the pathway.

F-3205

DEVELOPING AN ASSAY FOR MODELING PSYCHIATRIC DISORDERS IN VIVO

anna tarabrina, Krishnan Padmanabhan
LOG-G, Salk Institute, san diego, CA, United States

Many psychiatric disorders, including schizophrenia and autism spectrum disorders, present problems for investigation due to the difficulty of generating animal models that reflect the complex pathological, molecular, and genetic changes underlying the disorders. Murine models that target certain genes associated with these disorders have several downsides. The problems with animal models that target single gene may include: 1) some knockdown mice models are not viable, 2) altered gene is not the sole cause of the disorder, or if it is it represents just the first alteration from which other pathologies arise that could not be modeled by a single gene approach. The development of stem cell technology, including inducible pluripotent stem cells (iPSCs) isolated from patients has allowed progress to be made addressing the genetic causes of the disease. However, these approaches are not applicable for studying neuronal circuits.
This project proposes to develop an assay for studying human disease in mouse model systems by engrafting (transplanting) stem cells including neuronal precursor cells (NPCs) into the mouse and characterizing how they mature and integrate into existing circuits. This project is the first step in establishing a transplantation assay for NPCs into three different areas of the brain and then following this with examination and characterization of the possible neuronal differentiation and migration of transplanted NPCs. Preliminary data demonstrates that engrafted human NPCs can be successfully transplanted into the olfactory bulb and the cortex areas of the mouse brain. Transplanted human NPCs survived and differentiated into Tuj-1 (neuronal marker) positive cells. NPC survival was highly variable ranging from few cells per engraft to hundreds of cells.

F-3206

RNA-SEQ OF MONKEY EMBRYONIC STEM CELL DERIVED NEURAL ROSETTES REVEALS LONG NON CODING RNA INVOLVED IN NEUROGENESIS

Tao Tan[1], Shuang Ji[2], Bin Lu[1], Yuyu Niu[1], Weizhi Ji[1]
[1]Yunnan Key Laboratory of Primate Biomedical Research, Kunming, China, [2]Kunming Institute of Zoology,Chinese Academy of Sciences, Kunming, China

Long non-coding RNAs(lnc RNAs) are a newly discovered class of mRNA-like non-conding RNAs, which have been proposed to play a role in regulating the pluripotent state of embryonic stem cells. However until now little is known about how many and which long non-coding RNAs are play pivotal role in early neurogenesis. In this study , we address this using monkey embryonic stem cell as a model to determine lnc RNAs dynamics during early neurogenesis. Neural rosettes were derived from the monkey embryonic stem cells, and these cells expressed neural stem cell specific genes,such as Sox2 and Nestin. They also expressed Zo1, Plzf and Dach1, which only expressed in Neural rosettes cells. Intriguingly, Neural rosettes can differentiate toward CNS(Central Nervous System) and PNS (Peripheral Nervous System) fates in response to appropriate developmental signals. Through genome-wide expression analysis using RNA deep sequencing (RNA-Seq) technology, we compared the RNA expression profiles between embryonic and neural rosettes cells. We found dynamic changes in the expression of coding genes, long non-coding RNAs (lncRNAs) and splice isoforms during the transition from pluripotent stem cells to early neural rosettes. Moreover through bio-informatics analysis, we identified three lnc RNA may contributed to embryonic stem cells to neural rosettes transition.

F-3207

MAINTAINING THE POTENCY OF HUMAN SPINAL MOTONEURON PRECURSORS

Zhong-wei Du, Hong Chen, Hui-sheng Liu, Kun Qian, Su-Chun Zhang
Waisman Center, University of Wisconsin, Madison, WI, United States

Recent progress in cell-based modeling using human pluripotent stem cell (hPSC)-derived motor neurons (MNs) has opened new opportunities for understanding the development of the motor system, modeling MN disease and screening drugs. However, these studies are hindered by low and variable efficiency of MN generation from hPSCs as well as our inability to maintain the potency of lineage committed progenitors. By using a combination of small molecules in a chemical defined neural medium, we have guided human iPSCs to an enriched population (>90%) of OLIG2+ MN precursors in 12 days. More importantly, the OLIG2+ MN precursors can be maintained in a state of self-renewal for at least 10 passages, and upon differentiation the OLIG2+ MN precursors produce MNX1+ post-mitotic MNs in 1 week and CHAT+ mature MNs in 2 weeks. The MNs produced from the expandable precursors exhibit functional properties, including formation of neuromuscular junctions when co-cultured with skeletal muscle cells and projection of axons toward muscles when grafted into the developing chick spinal cord. Thus, a single iPSC can generate 107 MNs. These OLIG2+ MN precursors can be frozen and thawed with a recovery rate of more than 90%, which offers a consistent cell population for research and development. This model also opens an avenue for understanding the maintenance of lineage committed stem/progenitor cells.

F-3208

THE TRANSLATION REPRESSOR 4E-BP1 MEDIATES MTORC1 ACTION ON NEURAL STEM CELL DIFFERENTIATION AND NEURON PRODUCTION

Nathaniel W. Hartman, Tiffany Lin, David M. Feliciano, Grace Paquelet, Angelique Bordey
Neurosurgery, Yale University School of Medicine, New Haven, CT, United States

As the building blocks of the brain, neural stem cells (NSCs) determine the exact numbers of neurons generated for proper circuit function. NSCs can self-renew or terminally differentiate into duaghter cells that can amplify, eventually giving rise to neurons. One intracellular hub, the mammalian target of rapamycin (mTOR) signaling pathway, has emerged as a key player in NSC self-renewal. Dysregulations of mTOR signaling in NSCs is implicated in several neurological disorders associated with abnormal cell growth, impaired neurogenesis, and tumor formation. However, the function of mTOR on NSC self-renewal and the downstream regulatory mechanisms are ill-defined. Here, we found that genetically decreasing mTOR complex 1 (mTORC1) activity in neonatal mice resulted in reduced neural lineage expansion and aborted neuron production. This effect was observed only when mTOR activation is reduced in NSCs, preventing their differentiation during asymmetric self-renewal. Conversely, hyperactivation of mTORC1 induces differentiation of NSCs at the expense of self-renewal. Constitutive activation of the translation repressor 4E-BP1 rescued hyperactive mTORC1 induction of NSC differentiation. These data demonstrate the crucial role of mTORC1 and cap-dependent translation by 4E-BP1 in NSC differentiation during asymmetric self-renewal and neurogenesis in neonates. In addition, this research places 4E-BP1 at the center stage for drug discovery aiming at controlling mTORC1 function on NSC self-renewal in neurodevelopmental mTORopathies, such as tuberous sclerosis complex and Cowden syndrome, and conditions of decreased mTORC1 activity such hypoxia during perinatal life.

F-3211

NANOFIBERS FOR HUMAN PLURIPOTENT STEM CELL -DERIVED NEURAL CELLS

Mervi Ristola, Tiina Joki, Anu Hyysalo, Susanna Narkilahti
Institute of Biomedical Technology, BioMediTech, University of Tampere, Tampere, Finland

Background: Central nervous system (CNS) deficits usually cause severe dysfunctions to patients. Medical strategies that are nowadays used are not effective to many CNS deficits and the recovery from the deficits is poor due to the low regeneration capacity of nervous tissue. This has led to the idea of cell transplantation therapy as a potential future treatment. Challenging issues related to cell transplantations are poor survival and integration of transplanted cells to the host tissue. One option is the use of scaffolds that act as a supporting matrix for the cells and can provide a more hospitable environment for them. For example, the synthetic nanofiber scaffolds have been successfully transplanted into human patients improving their quality of life. One benefit of nanofibers is that they can be easily functionalized through encapsulation or immobilization of growth factors or extracellular matrix proteins. In neural applications, nanofiber scaffolds could help the survival of cells and guide cells towards wanted behavior and they are a promising approach for the reconstruction of injured CNS. The aim of this study is to test the suitability of nanofibers for in vitro culturing of human pluripotent stem cell (hPSC) -derived neural cells.
Materials and Methods: Commercially available electrospun polycaprolactone (PCL) nanofibers either aligned or randomly oriented were tested. hPSC-derived neurons, oligodendrocytes and astrocytes, differentiated by the method developed in our group, were cultured on the fibers, and the cytocompatibility and the attachment, growth and differentiation of cells were studied using immunocytochemical staining.
Results: Nanofibers were non-cytotoxic and immunocytochemical staining confirmed the presence of the neurons, oligodendrocytes and astrocytes on the nanofibers. Both aligned and randomly oriented nanofibers supported the growth of neurons, oligodendrocytes and astrocytes, and oligodendrocytes seemed to migrate along the aligned nanofibers most prominently.
Conclusions: According to our in vitro studies, nanofibers are suitable material for hPSC-derived neurons, oligodendrocytes and astrocytes supporting their growth and maturation. A combination of nanofiber scaffolds and hPSC-derived neural cells could lead to the development of a better strategy for the reconstruction of injured CNS.

F-3212

APICOBASAL POLARITY AND NEURONAL DIFFERENTIATION WITHIN EMBRYONIC STEM CELL-DERIVED NEURAL ROSETTES

Erin C. Banda, Anna McKinsey, Noelle D. Germain, Laura B. Grabel
Biology, Wesleyan University, Middletown, CT, United States

The neural stem cells (NSCs) of the embryonic and adult CNS are polarized, with apical and basal domains. This polarity is key to the regulation of neurogenesis. Embryonic stem cells (ESCs) undergoing neurogenesis *in vitro* also become polarized, forming
neural rosettes, in which differentiating NSCs are arranged radially around a central lumen. The apical domain, adjacent to the lumen, contains adherens junctions. Using a direct monolayer protocol for neural differentiation of human ESCs, we now show that during rosette formation, primary cilia translocate to the apical end of NSCs, projecting into the lumen, as observed for radial glia neural stem cells in the developing CNS that project primary cilia into the lumen of the neural tube or ventricles of the neocortex. Primary cilia serve an important role in Hedgehog signal transduction, with the Hedgehog effector Smoothened (Smo) translocating to primary cilia in responding cells. We demonstrate that in rosette cultures, Smo moves into primary cilia preferentially under Shh treated conditions compared to control conditions. In the absence of exogenous Hedgehog, the majority of Smo is present at the base of the primary cilia, while in Shh treated cultures Smo localizes to the axoneme. We also describe the presence of a basal cell surface on rosette NSCs, characterized by the localization of the extracellular matrix receptor β1 integrin.
In addition, as shown during neural development *in vivo*, we demonstrate a role for the polarity complex, including aPKC, Par6, and Par3, as well as Notch signaling in rosette neurogenesis. Par3 selectively localizes to the apical domain of rosette NSCs and treatment with aurothiomalate (ATM), a small molecule inhibitor that prevents formation of a functional polarity complex, disrupts the subcellular localization of Par3 and inhibits neuronal differentiation while increasing the proportion of NSCs. In contrast, Notch inhibition mediated by treatment with the gamma-secretase inhibitor DAPT leads to increased neuronal differentiation and depletion of the NSC population. Taken to-

gether, these data demonstrate that ESC-derived neural rosettes can serve as an *in vitro* model for studying neurogenesis.

F-3213

MODELING HUMAN NEURAL CREST DEVELOPMENT WITH PLURIPOTENT STEM CELLS.

Mirella Dottori[1], Mark Denham[1], Kouichi Hasagawi[2], Dong Zhang[3], Shelley Hough[1], Trevelyan Menheniott[3], Jessie Leung[1], Ben Rollo[3], Don Newgreen[3], Martin Pera[1]
[1]University of Melbourne, Parkville, Australia, [2]Institute for Integrated Cell-Material Sciences, Kyoto, Japan, [3]Murdoch Childrens Research Institute, Melbourne, Australia

Neural crest (NC) cells are multipotent stem cells that generate most lineages of the peripheral nervous system, as well as non-neural lineages including melanoctyes, cartilage and smooth muscle. In the embryo, specification of neural crest progenitors occurs soon after gastrulation, which can be challenging to study in human. However, this can now be addressed using human pluripotent stem cells as a cellular model of embryogenesis. We have previously described that inhibition of GSK3β and activin/nodal pathways in human pluripotent stem cells induces their differentiation to 'pre-neuroepithelial' progenitors, which express Sox2 and Otx2 but are negative for Oct4 and Pax6. We showed that exposure of sonic hedgehog to pre-neuroepithelial progenitors can efficiently generate floor plate cells. We now describe using the same chemically defined system to generate roof plate cells and NC. NC progenitors were able to differentiate into NC lineages in vitro and showed intrinsic migratory properties in vivo when transplanted in avian embryos. This study shows that this pre-neuroepithelial stage during neural induction of human pluripotent stem cells is a pivotal point that dictates their fate to major lineages of the nervous system.

F-3214

GENE EXPRESSION PROFILING IDENTIFIES CD10 AS A MARKER FOR MESENCHYME-COMMITTED NEURAL CREST PROGENITORS IN THE ADULT MAMMALIAN CAROTID BODY

Elena Navarro-Guerrero, Aida Platero-Luengo, José López-Barneo, Ricardo Pardal
Fisiología Médica y Biofísica, Instituto de Biomedicina de Sevilla (IBiS). Hospital Universitario Virgen del Rocío/CSIC/ Universidad de Sevilla., Sevilla, Spain

The carotid body (CB) is a neural crest-derived oxygen-sensing organ in the peripheral nervous system (PNS); responsible for the adaptation of the organism to hypoxemic situations like those suffered by high altitude residents or by patients with obstructive pulmonary disease. CB neural parenchyma is organized in clusters of neuron-like glomus, or type I cells, which constitute the chemoreceptor units that communicate low O2 conditions to afferent sensory nerve fibers. During a persistent exposition to hypoxia, the organ achieves functional adaptation by suffering structural changes including a profound angiogenic process and a clear increase in the number of neuronal type I cells. Our group has recently shown that this physiological neurogenesis depends on the proliferation and differentiation of a resident population of neural crest-derived progenitors, identified as glia-like (GFAP+) sustentacular, or type II cells. Consistently, these cells behave as neural stem cells in vitro, growing as neurospheres (NS) composed by nestin+ intermediate progenitors within the core, and tyrosine hydroxylase (TH) positive neuronal type I cells within differentiating blebs.
Little is known about the molecular mechanisms underlying CB stem cell (CBSC) proliferation and differentiation. We have set up conditions in vitro to enrich NS cultures in differentiated or undifferentiated cells, and have used microarray-based gene expression analysis on these NS to learn more about signalling pathways involved in the biology of CB progenitor cells. Microarray results were validated by confirming that proliferation genes are mainly expressed in undifferentiated conditions whereas neuronal differentiation genes are highly expressed in differentiated cultures. When interrogating this gene expression analysis for cell surface markers expressed in undifferentiated cells, CD10 was selected as the gene with the highest fold-change expression in proliferative conditions versus differentiated samples. CD10 is a zinc-dependent metalloendopeptidase that inactivates a number of signalling peptides. We found CD10 expression partially co-localizing with nestin and GFAP, suggesting the possibility

of using CD10 as a CB progenitor surface marker. Flow cytometry analysis has shown 30% of CB cells being positive for CD10, and sorting of these cells enriches several folds for neurosphere-forming activity. These results, in addition to the fact that we observed NS formed by CD10- cells, suggest that this marker identifies a subpopulation of CB progenitor cells. When studying differentiation of CD10+ progenitors in vitro, we have found that they can differentiate in adherent conditions into smooth muscle (SMA+) or endothelial cells (lectin+), but are not able to differentiate into TH+ neuronal cells. These results propose a mesenchyme-committed nature for CD10+ cells. We are currently performing cell-fate mapping experiments to position CD10+ progenitors within CBSC lineage. Moreover, we are also setting up functional assays in vitro to study the role of CD10 in the physiology of CB, considering the regulation of differentiation into mesenchymal lineage as one possible function. Understanding the mechanisms by which CD10 participates in regulating the biology of CBSCs might have important implications for the study of CB neurogenic niche and for the potential use of these neural progenitors in cell therapy.

F-3215

REGULATION OF NEURAL PROGENITOR CELL PROLIFERATION BY ANKHD1

Robert Hermann, Ana Martin-Villalba
Molecular Neurobiology, German Cancer Research Center (DKFZ), Heidelberg, Germany

During mammalian neural development, a wide variety of neurons and glial cells differentiate from common precursor cells. Postnatal, radial glia cells transform to adult neural stem / progenitor cells (NPCs). However, the mechanisms controlling NPC fate are not fully understood. Microarray analysis of ribosome-enriched transcripts in NPCs revealed a preferential loading of ribosomes with transcripts important for neuronal differentiation. One preferentially loaded transcript is Ankyrin repeat and KH domain containing 1 (Ankhd1). Ankhd1 is a 270kDa protein that contains multiple ankyrin repeats for protein-protein interactions as well as a KH domain important for RNA binding. It's ortholog in Drosophila, Mask, has been shown to be crucial for photoreceptor differentiation, proliferation and survival. Albeit, its function in neural progenitors remains elusive.
Here, we show that Ankhd1 is expressed throughout murine embryonic brain development and in adult neural progenitor cells. Expression can be detected as early as E12.5 and increases during the course of cortical development. Selective knockdown of Ankhd1 via in utero electroporation in the developing neocortex inhibits differentiation into neurons. Moreover, knockdown of Ankhd1 at embryonic day 14.5 enhanced NPC proliferation in vivo. Interestingly, Ankhd1 remains highly expressed in adult NPCs of the subventricular zone. Similar to NPCs during development, knockdown of Ankhd1 in primary neurospheres cultures of adult NPCs promotes their proliferation and inhibits neuronal differentiation. Conversely, overexpression of human Ankhd1 promoted neuronal differentiation of adult NPCs in vitro. Together, these results substantiate the relevance of Ankhd1 for controlling proliferation and differentiation of neural progenitors.

F-3216

NATO3 CONTROLS NEURAL STEM CELL DIFFERENTIATION DURING DEVELOPMENT OF THE VENTRAL NEURAL TUBE

Abed Al-Fatah M. Mansour[1], Sophie Zisman[1], Avihu Klar[2], Nissim Ben-Arie[1]
[A]Department of Cell and Developmental Biology, Institute of Life Sciences, [B]Department of Medical Neurobiology, Institute for Medical Research – Israel-Canada,, [1]Hebrew University of Jerusalem, jerusalem, Israel

During development
of the vertebrate central nervous system, diverse neural cell types are
generated from the neural tube, a simple group of neural stem cells. This
diversity is mainly coordinated by organizing centers, such as the floor plate
(FP), which is located in the ventral midline of the neural tube. FP cells
regulate dorso-ventral patterning, differentiation and axonal guidance by
secreting morphogens. These signaling cues induce the expression of multiple

combinations of transcription factors codes that determine cellular identities. Despite the importance of FP cells in neural development, the genetic and molecular programs that underline FP development are not fully understood. Here, we found that the spatio-temporal expression profile of the transcription factor Nato3 coincides with FP specification, suggesting a possible role in establishing FP fate. Ectopic expression in the chick neural tube demonstrate that Nato3 is sufficient to induce FP-like cells, as assayed by the ectopic expression of Foxa2. These FP-like cells altered their normal fate, leading to a change in the patterning profile in the developing spinal cord. Complementary, in Nato3 null mice, FP specification was severely disrupted. Taken together, Nato3 is suggested to be a novel determinant factor that control differentiation and maintenance of FP cells during neural tube development.

F-3217

DIFFERENTIATION OF CHOLINERGIC NEURONS FROM HIPS CELLS AND RESTORATION OF COGNITIVE FUNCTION BY THEIR TRANSPLANTATION IN MICE WITH DEMENTIA

Narutoshi Fujiwara, Kenji Takai, Erika Takada, Chieko Hirotsu, Nagisa Arimitsu, Asako Saito, Jun Shimizu, Noboru Suzuki
Department of Immunology and Medicine, St. Marianna University School of Medicine, Kawasaki, Japan

[Purpose] Dementia is a memory loss disorder, which is caused by a variety of brain diseases and results from a gross disruption of neural cells, including cholinergic and GABAergic neurons. Specifically, cholinergic dysfunction is strongly associated with both behavioral and physiological symptoms. Radical cures have not been developed at present. Therefore, neural cell transplantation to replace lost neurons is a prospective therapeutic approach as treatment of dementia.
In this study, we tried to generate forebrain neurons, the majority of which consisted of cholinergic neurons, from human iPS (hiPS) cells and to transplant these cells into the bilateral hippocampal lesions of APP transgenic mice (PDAPP). PDAPP showed significant hippocampal atrophy at 3 months of age.
[Materials and methods] (Culture of hiPS cells) A hiPS cell line was obtained from RIKEN Cell Bank (cell designation: 253G1) and was maintained according to RIKEN cell preparation manual. (Cell differentiation) We developed embryonic bodies (EB) from undifferentiated hiPS cells by 4-day floating culture (from day 0 to day 4). Then EB were replated onto fibronectin-coated dishes on day 4. After 24 hours, medium was switched to differentiation medium that consisted of DMEM/F12 supplemented with N2. We added retinoic acid (RA), sonic hedgehock (SHH) and noggin (NOG) twice (on day 5 and day 7) and cultured for up to 11 days (from day 8 to day 19). We performed immunocytochemistry to evaluate the differentiation of these cells. (Transplantation and Morris water maze test) Neural cells derived from hiPS cells were collected and suspended into ice-cold PBS at the density of 2 x 105/2µl. 10-week-old PDAPP mice were injected with 2µl of a suspension (n=7) or vehicle (n=10) into the bilateral hippocampus. Learning and memory functions were evaluated by Morris water maze (MWM) before and after the transplantation. Histology and immunohistochemistry were performed on 45 days after the transplantation.
[Results] (Cell differentiation) Approximately 65 % of the differentiated cells were human neural cell adhesion molecule (hNCAM) positive and they expressed mRNA of chorine acetyl transferase, beta III tubulin, nestin and neurofilament. They were stained with several anti-neuron associated protein antibodies on day 8 and 19. The cells expressed Nestin on day 8, and its expression disappeared on day 19. These cells expressed choline acetyltransferase on day 19. (Transplantation and MWM test) Transplanted hiPS cell derived neural cells were localized in the bilateral hippocampal lesions and these cells expressed hNCAM. Before transplantation, neural cell transplanted PDAPP mice required 69.1±10.6 seconds and vehicle-transplanted PDAPP mice required 89.7±0.2 seconds to find the respective hidden platform ($P > 0.01$). By contrast, transplanted mice engaged less time to find the respective hidden platform (26.5±8.0 seconds) compared with vehicle-transplanted mice (72.8±8.4 seconds) after transplantation ($P < 0.01$). Differences between data were assessed by using the Student's two-tailed t test for independent data.

[Conclusions] Exposure of EB to RA, SHH and NOG enriches for neural cells that can be further differentiated into post mitotic cholinergic neuron. Moreover, transplantation of these cells improved spatial memory dysfunction of PDAPP mice. We infer that hiPS derived neural cell transplantation to replace lost neurons is a prospective therapeutic approach to the treatment of dementia.

F-3218

ISOLATION AND PURIFICATION OF NEURALLY DIFFERENTIATED CELLS DERIVED FROM MSIPS CELL LINES

Daniella Herszfeld[1], Guizhi Sun[2], Aude Sylvain[2], Natalie Payne[2], Martin Short[2], Claude Bernard[2]
[1]Monash University, Clayton, Melbourne, Australia, [B]ARMI, [1]Monash University, Clayton, Melbourne, Australia

The introduction of technologies capable of reprogramming human somatic cells into induced pluripotent stem (iPS) cells offers a unique opportunity to study many aspects of neurodegenerative diseases in vitro. This could ultimately lead to novel drug development and testing for these severe neurological disorders. We recently reported that human dermal fibroblasts from a patient with relapsing-remitting Multiple Sclerosis (MS) were reprogrammed to pluripotency by retroviral transduction using defined factors (*OCT4, SOX2, KLF4*, and *c-MYC*). The MSiPS cell lines resembled human embryonic stem (hES) cell-like colonies in morphology as well as gene expression and exhibited silencing of the retroviral transgenes after four passages. The MSiPS cells were successfully differentiated into mature astrocytes, oligodendrocytes and neurons.
We have extended that study by generating additional iPS cell lines from MS patients displaying various stages of the disease and their healthy siblings as controls. The neural differentiation potential of those iPS cell lines was assessed.
In order to isolate and purify heterogeneous neural cell populations that were obtained from the differentiated MSiPS cell lines, a panel of antibodies was used to determine their surface markers signature.
Following two weeks of neural induction of MSiPS, we generated NSCs that could not only be propagated for many passages but could also be differentiated into a mixture of neurons and glial cells, as indicated by Immunofluoresence and PCR analysis.
The differentiated neural cells were subsequently sorted by FACS to obtain two distinct purified populations of neural cells, namely neurons and astrocytes. The neuron population was CD184-/CD44-/CD15 low /CD24+ and expressed mature and subtype specific neuronal markers. The astrocytes were CD184+/CD44+ and expressed the astrocyte marker GFAP.
This strategy provided us with highly purified population of neurons and glia cells, thus enabling downstream studies that require consistent and defined neural cell populations.
Current studies are underway to ascertain potential genetic and physiological differences that may exist between MS patients and their healthy sibling controls.

F-3221

HIGHLY EFFICIENT AND ROBUST GENERATION OF NEURAL STEM CELLS FROM HUMAN PLURIPOTENT STEM CELLS USING A CHEMICALLY DEFINED NEURAL INDUCTION MEDIUM

Yiping Yan, Soojung Shin, Alexandria Sams, David Kuninger, Mohan Vemuri
Primary & Stem Cell Systems, Life Technologies, Frederick, MD, United States

Human pluripotent stem cells (hPSCs) including human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) are excellent sources for studies of cell fate specification, disease modeling and drug screening. In order to produce various neural cells from hPSCs, the induction to neural stem cells (NSCs) is the first important step. Conventional methods of NSC derivation from hPSCs typically involve in embryoid body (EB) formation or co-cultures with stromal cell lines, which have several disadvantages including time-consuming protocols, variable quality of derived NSCs and contamination with non-neural cells. There is a critical need for robust, streamlined and scalable workflows for generation of NSCs from hPSCs. We have developed a chemically defined neural induction medium which can convert hPSCs into NSCs in one week with 80-90% of efficiency but without the time con-

suming, laborious processes of embryoid body formation and mechanical NSC isolation. Human PSCs maintained under feeder-free conditions or on mouse embryonic fibroblasts (MEFs) underwent NSC induction with similar efficiencies. Briefly, following hPSC subculture and growth to 10-20% confluence, growth medium was exchanged to neural induction medium and replaced every other day. By day 7 of neural induction, the total cell number typically increased by 30-40 times. To confirm the phenotype of derived cells, immunofluorescent staining for pluripotent marker Oct4 and neural markers including Sox1, Sox2 and Nestin was performed. The results showed that fewer than 1% of cells expressed Oct4 and 80-90% of cells were positive for Sox1. The percentage of Sox2 and Nestin positive cells exceeded 95%. Further experiments showed that derived cells can be expanded 8-10 fold with each passage. The expanded cells maintained phenotype, exhibited normal karyotype and can be differentiated into neurons, astrocytes and oligodendrocytes. These results suggest that the derived cells from hPSCs are truly NSCs. Microarray analysis showed a 75% overlap in gene expression profiles between human fetal NSCs and those derived using our neural induction medium. In addition, neural induction medium worked for multiple hPSC lines including hESC and hiPSCs generated either by episomal vector or Sendei Virus (Cytotune™) mediated delivery of reprogramming factors, with similar neural induction efficiency. In conclusion, we demonstrate the development of a new chemically defined neural induction medium enabling efficient generation and expansion of NSCs from hPSCs.

F-3222

TRANSPLANTATION OF NEURAL PRECURSOR CELLS WITH TRANSGENIC OVER EXPRESSION OF IL-1 RECEPTOR ANTAGONIST RESCUE MEMORY AND NEUROGENESIS IMPAIRMENTS IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

Ofra Ben Menachem Zidon[1], Yair Ben Menahem[1], Tamir Ben Hur[2], Raz Yirmiya[1]

[1]Psychology, The Hebrew University of Jerusalem, Jerusalem, Israel, [2]Neurology, Hadassah-Hebrew University Hospital, Jerusalem, Israel

Over the last two decades it became evident that inflammatory processes, including activation of microglia and production of pro-inflammatory cytokines, play an important role in the etiology and progression of Alzheimer's disease (AD). More specifically, over-production of the pro-inflammatory cytokine interleukin -1(IL-1) has been particularly implicated in the pathophysiology of AD. In the present study we used mice with transgenic over-expression of a mutant Amyloid Precursor Protein (sweAPP) (Tg2756 mice) to examine the role of brain IL-1 in mediating the disturbances in hippocampal-dependent memory (tested in the water maze and fear conditioning paradigms) and hippocampal neurogenesis exhibited by these mice. To achieve long-term inhibition of central IL-1 signaling mice were transplanted into the hippocampus with neural precursor cells (NPCs) derived from newborn mice with transgenic over expression of IL-1 receptor antagonist (IL-1raTG). NPCs derived from WT mice or sham transplantation served as controls. To assess the net effect of IL-1 blockade (not in the context of NPCs transplantation) we also examined the effects of chronic (4 weeks) intra-cerebroventricular (i.c.v.) administration of IL-1ra. We report that transplantation of IL-1raTG NPCs completely rescued the disturbances in hippocampal-dependent contextual and spatial memory as well as in neurogenesis. It also increased the number of endogenous hippocampal cells expressing the plasticity-related molecule BDNF. Similar, but less robust effects were also produced by transplantation of WT NPCs and by i.c.v. IL-1ra administration. NPCs transplantation produced alterations in hippocampal plaque formation and microglia density, which were not correlated with the cognitive effects of this procedure. The results provide the first example for the use of genetically manipulated NPCs in the treatment of AD, indicating that combination of NPCs therapy with delivery of the anti-inflammatory cytokine IL-1ra can be a viable and effective therapeutic procedure for AD-associated cognitive disturbances.

F-3223

NEURAL DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

Wade Johnson, Hanna Lentsch, Joy L. Aho

R&D Systems, Minneapolis, MN, United States

Neural progenitor cells (NPCs) have the ability to give rise to neurons, astrocytes, and oligodendrocytes. These cells have great therapeutic potential for a variety of neurological disorders, including neurodegenerative diseases. The availability of NPCs is limited and thus differentiation of these cells from pluripotent stem cells may provide an unlimited source for use in research and therapeutics. Here we used combinations of the small molecule inhibitors of TGF-β receptors (SB 431542), GSK-3β (BIO), and ROCK (Y-27632) for the differentiation of human pluripotent stem cells into neural progenitor cells. Neural progenitor cells were subsequently characterized by immunocytochemistry and flow cytometry using antibodies to detect progenitor cell markers, as well as their ability to differentiate into downstream derivatives.

F-3224

EMBRYONIC BRAIN ENDOTHELIAL CELLS AND NEURAL PROGENITOR CELLS PROMOTE RECAPITULATION OF A NEUROVASCULAR NICHE IN VITRO VIA RECIPROCAL SIGNALING

Ravi R. Vissapragada, Mauricio Contreras, Ajith Thomas
Surgery, Beth Israel Deaconess Medical Center, Boston, MA, United States

Introduction: The interactions between endothelial cells and neural progenitor cells (NPC) have been well documented in the literature. These interactions emerge from both direct cell-cell contact as well as autocrine/paracrine feedback signals. However, most studies have focused on coupling adult endothelial cells and NPC. Given that neurovascular development is most robust in the embryonic brain, we hypothesized that pairing embryonic brain endothelial cells (PvEC), known to be closely associated with neural proliferation and migration, with NPC would represent the ideal partners to renew regenerative processes in the injured brain.
Results: PvEC were isolated from brains of embryonic mice (E15). Immunohistochemistry with antibodies against endothelial cell markers showed expression of CD31/PECAM, CD144/VE Cadherin, and Nestin. A colorimetric assay (MTS assay) was conducted for measuring NPC proliferation. NPC proliferation was highest when in transwell co-culture with PvEC, when compared to baseline NPC proliferation and NPC transwell co-culture with adult brain endothelial cells (ABEC). Immunohistochemistry performed on NPC and PvEC transwell co-cultures at 3 and 7 days revealed that PvEC prolonged NPC's progenitor phenotype seen by high expression of Nestin and low expression of ß-tubulin III at day 3. In a cord formation angiogenesis assay, PvEC showed the highest potential for primitive vessel formation (measured as cord length), compared to ABEC and dermal microvascular endothelial cells (DMVEC) in basal conditions. PvEC in direct co-culture with NPC had yet higher cord length values and formed a structurally appreciable neurovascular network in vitro. PCR experiments studying expression of VEGFa corroborated these results where PvEC in co-culture with NPC had significantly higher upregulation.
Conclusion: PvEC exhibited a range of extraordinary properties, including promoting NPC proliferation, delaying NPC differentiation, preferentially forming premature vessels, and upregulating VEGF in presence of NPC. Our data strongly suggests that PvEC and NPC communicate through different channels, ranging from soluble factor exchange to direct cell-cell contact. Understanding these mechanisms of communication between NPC and PvEC may have a significant role in recreating the embryonic milieu in the injured brain through use of either cellular transplants or formulations of secreted factors.

F-3225

NEURAL STEM AND PROGENITOR CELLS REQUIRE THE SPECIFICITY PROTEIN 2 FOR REGULATION OF CYTOKINESIS LENGTH

Huixuan Liang
CVM, MBS, North Carolina State University, Raleigh, NC, United States

Timely and step-wise transitions through complex stages of mitosis are critical to integrity and fate of stem cell divisions. Here we demonstrate that neural stem cells employ a putative zinc-finger transcription factor, Specificity

Protein 2 (Sp2), as a key regulator of M-phase progression. Cell autonomous function of Sp2 during the cytokinetic stages of the M-phase was revealed using time-lapse imaging of neural stem cells genetically labeled at clonal densities in vivo and in vitro. Specific defects were detected during the intercellular bridge formation, centrosomal motility events, and subsequent abscission to complete cytokinesis. Analysis of the Sp2-fused reporters confirmed its localization to the nucleus, but surprisingly revealed that Sp2 is also a stable component of the centrosomal complex. Sp2-associated centrosomes transited from perinuclear loci to the intercellular bridge during cytokinesis. Conditional deletion of Sp2 results in excessive shuffling of centrosomes to and from the intercellular bridge during cytokinesis and abscission failure. Further molecular characterization revealed that the N- and C-domains of Sp2 contain distinct subcullar localizing signals and are both required for successful cytokinesis. To utilize a platform where stem cell mitoses and lineal progression of their offspring are molecularly and anatomically well-defined, we conditionally deleted Sp2 in the cerebral cortices. This approach revealed that Sp2-defective cytokinesis severely disrupts fate decisions in neural stem cells. Specifically, loss of Sp2 severely interrupted the normal switch in developmental potential from proliferative to neurogenic divisions during embryonic and perinatal periods. These findings lead to the exciting concept that regulation of the M-phase and cytokinesis in particular potentially impact symmetric and asymmetric decisions in neural stem cells in an Sp2-dependent manner.

F-3226

A NOVEL ROLE FOR AUTISM-ASSOCIATED ANKRD11 IN THE DEVELOPING CEREBRAL CORTEX.

Denis J. Gallagher[1], Sarah Burns[1], Annie Paquin[1], Alexa Bramall[1], Gordon Keller[2], David Kaplan[1], Freda Miller[1]
[1]Developmental and Stem Cell biology, The Hospital for Sick Children Toronto, Toronto, ON, Canada, [2]McEwen Centre for Regenerative Medicine, Toronto, ON, Canada

Increasing evidence suggests that perturbations in stem cell differentiation during the development of the nervous system may underlie cognitive dysfunction associated with neurodevelopmental disorders. In that regard, we have identified a novel role for a recently identified autism-associated gene, ankyrin repeat domain-containing protein 11 (Ankrd11), in the development of the cerebral cortex. Ankrd11 is a nuclear protein capable of regulating transcription through recruitment of histone-modifying enzymes such as histone deacetylases (HDACs), but its physiological role is still unknown. Here, we show that Ankrd11 is expressed in both embryonic cortical precursors and in newly-born cortical neurons. We demonstrate that genetic knockdown of Ankrd11 decreases proliferation and inhibits neurogenesis in cultured cortical precursors. Embryonic cortical precursor proliferation and differentiation were similarly perturbed after acute shRNA-mediated knockdown of Ankrd11 *in vivo* using *in utero* electroporation. Furthermore, “Yoda” mice, which are heterozygous for a point mutation in the C-terminal, HDAC-binding domain of Ankrd11 display cortical defects. Overexpression of HDAC3 rescues the effects of Ankrd11 knockdown on proliferation, suggesting that Ankrd11 mediates its effects in cortical precursors by recruiting and regulating HDACs. To ask whether Ankrd11 is similarly important in human cortical precursors, we developed a method for generating forebrain neural precursors from human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs). Knockdown of Ankrd11 in these human precursors causes the same deficits in proliferation and neurogenesis as seen in the rodent neural precursors. Thus, Ankrd11 is a transcriptional coregulator that controls both rodent and human neural stem cell biology. Moreover, our data support the idea that deficits in neural stem cell biology underly at least some of the cognitive deficits seen in humans with autism spectrum disorder as a consequence of mutations in the Ankrd11 gene.

F-3227

INJURY SIGNALS INCREASE NEURAL STEM CELL FATE POTENTIAL

Enric Llorens Bobadilla, Sheng Zhao, Robert Hermann, Gonzalo Saiz Castro, Ana Martin-Villalba
Molecular Neurobiology, German Cancer Research Center (DKFZ), Heidelberg, Germany

During the development of the central nervous system, multipotent neuronal progenitors follow a precise differentiation process to generate the exquisite variety of neuronal subtypes present in the mature brain. Unlike em-

bryonic progenitors, adult neural stem cells (NSCs) only give rise to restricted neuronal subtypes, namely inhibitory interneurons in the olfactory bulb and glutamatergic granule neurons in the hippocampus. However, after brain injury this fate restriction can be partially overcome. The factors of the 'injury niche' that positively influence neurogenesis and that may be responsible for fate reprogramming remain largely unknown. Here we study the regulation of NSCs plasticity by CD95L, a cytokine of the TNF family that is specifically upregulated after brain injury. Using heterochronic transplantations into the developing brain as a measure of fate potential, we show that only upon CD95L stimulation adult NSCs migrate and differentiate into cortical and hippocampal regions. Genome-wide transcriptional profiling revealed that CD95L selectively regulates genes involved in neuron development and chromatin remodeling, suggesting epigenetic priming. Accordingly, ChIPseq for several histone modifications uncovers that multiple loci gain active chromatin marks after stimulation, probably facilitating transcriptional activation during neuronal subtype-specific differentiation. Altogether, we show that cues from the injury niche promote a permissive chromatin state that endows adult NSCs with increased fate plasticity.

F-3228

SYNDECAN-1 AND P38GAMMA INSTRUCT THE LINEAGE PROGRESSION FROM RADIAL GLIAL CELLS TO INTERMEDIATE PROGENITOR CELLS DURING MAMMALIAN CEREBRAL CORTICAL DEVELOPMENT

Qingjie Wang, Landi Yang, Sally Temple
Neural Stem Cell Institute, Rensselaer, NY, United States

The transition from multipotent stem cell to transit amplifying cell is a critical first step in lineage progression during the generation of many tissues, including forebrain. For example, in the developing cerebral cortex, multipotent apical radial glial cells (RGCs) in the ventricular zone (VZ) give rise to transit amplifying intermediate progenitors (IPCs) that form the subventricular zone (SVZ). The size of the SVZ and the level of transit amplification are critical factors determining the thickness of cortical layers, and have been proposed to dictate the evolution of cortical size in mammals. The mechanisms underlying lineage progression from RGC to IPC fates are still largely unknown. In previous studies, we showed that the heparan sulfate proteoglycan Syndecan-1 (Sdc1) is highly expressed in the germinal niche in the developing mouse cerebral cortex. Acute knockdown of Sdc1 causes premature differentiation of cortical progenitor cells, demonstrating its key role as an environmental regulator (Wang et al, 2012). Here we report that overexpression of Sdc1 in the developing cortical niche in vivo promotes the switch in progenitor fate from RGCs to IPCs. We found that exogenous Sdc1 activates p38gamma (MAPK12) to promote the RGC-IPC switch, an effect rescued by knocking down p38gamma. Consistent with this, overexpression of p38gamma alone can promote the RGC-IPC fate switch, while knocking down p38gamma in vivo impairs cortical neurogenesis by reducing expression of Tbr2, a key determinant of the IPC fate. Together, these data show that Sdc1, an essential niche signaling molecule, acts via the downstream signaling molecule p38gamma to regulate lineage progression from neural stem cells to the transit amplifying state in the developing mammalian cortex.

F-3231

NOTCH2 EXPRESSION AS A METHOD FOR IDENTIFYING UNDIFFERENTIATED MOUSE AND HUMAN NEURAL STEM CELLS

Ge Ma, Howard Mostowski, Brent McCright
CBER/OCTGT/DCGT, FDA, Bethesda, MD, United States

v:textbox>Neural stem cells isolated from fetal and adult tissues are being studied as possible sources for cellular therapies aimed at treating neurodegenerative diseases. A variety of methods have been used to generate these Neural Stem Cells (NSCs) and currently there are limited biomarkers available that accurately measure the developmental status of the cells. In this study, we investigated the potential of using Notch2 expression as a method for identifying undifferentiated mouse and human neural stem cells (NSCs). mNSCs were isolated from whole brain tissue of the mouse embryo day 14 (E14) or the sub-ventricular zone (SVZ) of adult mice, and H9 derived hNSCs were purchased from Invitrogen. The mice used as the mNSC source expressed GFP under the control of the Notch2 pro-

moter. Using an anti-Notch2 antibody, we confirmed that Notch2 co-localizes with EGFP+ cells in the subventricular zone (SVZ) of third ventricular (3V), fourth ventricular (4V) and lateral ventricular (LV) areas in mouse brain sections of E14 and adult, in areas that have been previously identified as NSC sources. By using flow cytometry and immunohistochemistry, we found that Notch2 expression directly correlates with features normally associated with both mouse and human NSCs. The cell surface staining of APC-conjugated-Anti-Notch2 for human NSCs (n=11, 41.33% positive) was significant higher than differentiated cells (n=6, 20.97% positive) with flow cytometry analysis. Notch2 expressing cells more efficiently form neurospheres and can differentiate into multiple cell types. We found that Notch expression is turned off much more rapidly than Nestin during differentiation. We also show that Notch2 expression decreases with passage number and that this correlates with a general decrease in the Notch activation as indicated by the reduction in Hes5 expression. Finally, we show that the level of Notch expression decreases during the differentiation of H9 derived hNSCs.
Conclusions: We postulate that Notch2 expression or Notch activation may be used to identify undifferentiated mouse and human neural stem cells.

F-3232

NSCS MIGRATION TOWARD BTSCS IN VITRO AND IN VIVO

shuang liu, Feng Yin, Wenhong Fan, Jianning Zhang, Ming Fan
Navy general hospital, Beijing, China

Objective To clarify the capacity of selective migration of human neural stem cells (hNSCs) to brain tumor stem cells (BTSCs) derived from glioblastoma, we studied the tropism of hNSC to BTSCs in vitro and in vivo. **Methods** We derived BTSCs from GBM tissues by neurosphere culture method, then, investigated the tropism ability of hNSCs to BTSCs using cell migration assay by transwell chamber in vitro and tumor tropism studies in the brain of nude mice. **Results** The BTSCs could be obtained from human GBM tissues, and formed tumorshpheres after cultured 7d in vitro. Ninety percenty of the cells in the tumorsphere were CD133 positive and nestin positive. Theses CD133 positive BTSCs could regenerate a GBM tumor in vivo when implanted intraventricularly. Cell migration assay showed that BTSCs have the capacity to actively attract hNSCs. The fate of hNSCs in vivo can be visualized under confocal fluorescence microscope. hNSCs exhibit extensive tropism toward the site of the tumor and infiltrate tumor foci . **Conclusions** BTSCs could be derived rapidly from GBM tissues by neurosphere method. The normal hNSCs possess the capacity to migrate toward BTSCs in vitro and in vivo. The tropism of hNSCs towards brain tumors may provide an additional tool for the treatment of brain cancer.

F-3233

NEUROPROTECTIVE EFFECTS OF COENZYME Q10 IN NEURAL STEM CELLS INJURED BY AMYLOID-BETA THROUGH ACTIVATION OF THE PI3K PATHWAY

Seong-Ho Koh, Young Seo Kim, Hojin Choi, Hyun Hee Park, Kyu-Yong Lee, Young Joo Lee, Seung Hyun Kim
Neurology, College of Medicine, Seoul, Korea, Republic of

Neurogenesis in the adult brain is important for memory and learning, and the alterations in neural stem cells (NSCs) may be an important part of Alzheimer's disease (AD) pathogenesis. The phosphatidylinositol 3-kinase (PI3K) pathway has been suggested to play an important role in neuronal cell survival and is highly involved in adult neurogenesis. Recently, coenzyme Q10 (CoQ10) was found to affect the PI3K pathway. We investigated whether CoQ10 could restore $A\beta_{25\text{-}35}$ oligomer-inhibited proliferation of NSCs by focusing on the PI3K pathway. To evaluate the effects of CoQ10 on $A\beta_{25\text{-}35}$ oligomer-inhibited proliferation of NSCs, NSCs were treated with several concentrations of CoQ10 and/or $A\beta_{25\text{-}35}$ oligomers. BrdU and Colony Formation Assays showed that NSC proliferation decreased with $A\beta_{25\text{-}35}$ oligomer treatment, but combined treatment with CoQ10 restored it. Western blotting showed that CoQ10 treatment increased the expression levels of p85α PI3K, phosphorylated Akt (Ser473), phosphorylated glycogen synthase kinase-3β (Ser9), and heat shock transcription factor, which are proteins related to the PI3K pathway in $A\beta_{25\text{-}35}$ oligomers-treated NSCs. To confirm a direct role for the PI3K pathway in

CoQ10-induced restoration of proliferation of NSCs inhibited by $A\beta_{25-35}$ oligomers, NSCs were pretreated with a PI3K inhibitor, LY294002; the effects of CoQ10 on the proliferation of NSCs inhibited by $A\beta_{25-35}$ oligomers were almost completely blocked. Together, these results suggest that CoQ10 restores $A\beta_{25-35}$ oligomer-inhibited proliferation of NSCs by activating the PI3K pathway

F-3234

THE EFFECT OF KV1.3 ION CHANNEL BLOCKER ON STEM CELLS NEURAL DIFFERENTIATION

Yuye Zhou, Guoqiang Hou, Zhiyuan Li
Guangzhou Institutes of Biomedicine and Health,Chinese Academy of Sciences, Guangzhou, China

In adult mammalian brain, multi-potent and self-renewing neural stem cells (NSCs) have the capacity to generate new neurons, astrocytes and oligodendrocytes. Thus, NSCs may function as a regenerative tool to replace the damaged neurons in response to neuron injury caused by inflammatory, brain injury or other reasons. However, the utilized potency is limited for their low differentiation efficiency. As part of signal messengers, membrane ion channels play crucial roles in biological signal conducting so that affecting the cell proliferation, differentiation and apoptosis in certain level. The functional roles which ion channels play in NSCs or their maturation process are seldom clear. Kv1.3 channel (potassium voltage-gated channel, shaker-related subfamily, member 3) was recently found to rescue the neuron loss caused by activated T-cells in neuro-inflammation diseases. Furthermore, selective blockade of Kv1.3 channel was also reported to promote the proliferation of neuron stem cells.
This project aims to explore the role of Kv1.3 channel on the differentiation progress from stem cells especially NSCs. The Kv1.3 specific blocker Psora-4, a small molecule named 5-(4-Phenylbufoxy) psoralen, was synthesized by chemical methods and applied to effect on NSCs or ESCs (embryo stem cells) differentiation. The neural stem cells were dissociated from the fetal mouse brain (13.5d) and differentiated in monolayer after cell subculture.
The study showed that the ratios of neurons differentiated from both NSCs and ESCs are up-regulated in the presence of Psora-4 (10nM). The percentage of neuron in NSCs progeny increased from 30% to 37% ($p<0.01$, $n=3$) and showed a concentration dependent manner. Furthermore, the percentage of possible mature and integrated neuron candidates with burly and long neuritis was also increased by 80% ($p<0.001$, $n=4$) compared to the control group. Besides, the efficiency of ESCs to NPCs (neuron progenitor cells) was increased by 13%. The neuron cells were characterized by both immunecytofluorescent with specific marker includes β-III-tubulin, Synapsin and Map2, and patch clamp technique which showed that neuron-like cells in d14 (from the day of alter the culture medium to differentiating medium) could fire continuous spontaneous action potential. The results of fluorescent quantitation real-time PCR showed that the mRNA level of Kv1.3 increased over time in differentiation, while insignificantly changed in the presence of Psora-4. Knocking down Kv1.3 by shRNA caused the decreasing of differentiation efficiency, which is contrary to Psora-4. Besides, Psora-4 made no impaction on the differentiation efficiency of Kv1.3 knocking down NSCs.
Taking together, we found that the Kv1.3 specific blocker may enhance the efficiency of stem cells neuron differentiation and ion channels play essential roles in neurogenesis.

F-3235

TRANSPLANTATION OF NGN2-OVEREXPRESSING HUMAN NEURAL STEM/PROGENITOR CELLS INTO HYPOXIC-ISCHEMIC INJURED BRAINS OF NEWBORN MICE

Kook In Park[1], Kyo-yeon Koo[2], Miri Kim[1], Kwangsoo Jung[1], Il-Shin Lee[3], Il-Sun Kim[3]
[A]The BK 21 Project for Medical Sciences, Pediatrics, [B]Department of clinical Genetics, [C]Department of Pediatrics, [1]Yonsei Univ College of Medicine, Seoul, Korea, Republic of

Hypoxic-ischemic (HI) brain injury in newborn infants represents a major cause of mental retardation, sensory neural hearing loss, blindness, cerebral palsy, epilepsy, learning disabilities or even death. Perinatal HI brain injury occurs in 2~4 of 1000 live term births, and ~60% of occurring preterm infants sustain permanent brain damage. Al-

though the regeneration of the central nervous system (CNS) has previously been considered to be very limited, recent studies suggest that neural stem/progenitor cells (NSPCs) have significant therapeutic potentials for incurable neurological diseases. NSPCs are defined as self-renewing and primordial immature cells with the capacity to give rise to differentiated progeny within diverse neural lineages in all the regions of the neuroaxis. However, most of implanted NSPCs reportedly remained to be undifferentiated state or differentiated into glial cells within the damaged CNS. Therefore, to replace injured neural cells, the transplantation of pre-differentiated cells could be expected to be more helpful.
Ngn2 is a family of basic helix-loop-helix, which is known to proneural gene which is critical regulator of neurogenesis through a neuronal specific signaling pathway associated with a Notch signaling during cortical development. In order to examine the effect of Ngn2 on the fate of human NSPCs (hNSPCs), we infected cells with adenoviral vector encoding Ngn2. Under the differentiation condition *in vitro,* more than 80-90% of Ngn2-expressing hNSPCs expressed early neuronal or oligodendroglial markers. To investigate the therapeutic potentials of pre-differentiated hNSPCs, we induced focal HI brain injury in 7-day postnatal CD1 pups, and then injected Ngn2-expressing hNSPCs (Ad-Ngn2-hNSPCs group), GFP expressing hNSPCs (Ad-GFP-hNSPCs group) or H-H buffer (vehicle group) directly into injured brain region of mice at 7 days after the induction of HI injury. When mice brains were analyzed at 6 weeks following transplantation, Ngn2-expressing donor-derived cells showed robust engraftment and migration within the regions of HI-injured brains, and preferentially differentiation into neurons. To evaluate the axonal sprouting, biotinylated dextran amine (BDA) was injected into the forelimb sensorimotor cortex of the ipsilateral cerebral hemisphere to HI brain injury at 9 weeks post-transplants, and then the animals were sacrificed 2 weeks later. Compared to Ad-GFP-hNSPCs or vehicle group, mice in Ad-Ngn2-hNSPCs group represented increased BDA-labeled fiber density in the contralateral dorsal funiculus of the spinal cord at C5 level, which suggests the enhanced axonal sprouting of the corticospinal tract in Ad-Ngn2-hNSPCs group. In addition, mice in Ad-Ngn2-hNSPCs group showed the prominently improved neurological and motor functions at 3, 5 and 7 weeks post-transplants, and sensory function at 7 weeks post-transplants compared to other groups. These results suggest that Ngn2-expressing hNSPCs could be a potentially therapeutic tool for neonatal hypoxic-ischemic brain injury.

F-3236

SUBSTANCE P ENHANCED REPAIR FROM ISCHEMIC BRAIN INJURY BY PROMOTING VESSEL REGENERATION

Eunkyung Chung
Kyung Hee University, Yong-in, Korea, Republic of

Previously, we have reported that substance P (SP) promotes wound repair by mobilization of mesenchymal stem cells and anti-inflammatory roles in the injured lesion. In this study, we determined the effect of SP in the experimental ischemic brain injury model, transient middle cerebral artery occlusion (tMCAO). SP prevented the expansion of infract volume near subventricular zone determined by TTC and silver staining of whole brain. In addition, enhanced expression of SMI-71, endothelial blood brain barrier (BBB) specific antigen, was observed in the SP-treated ipsilateral ischemic penumbra region. Regenerated SMI-71 blood vessels were significantly increased both in numbers and lengths. Moreover, SMI-71 was well co-localized with Glial fibrillary acidic protein (GFAP)-positive astrocytes, which represent maturation of BBB. These results suggest that intravenous treatment of SP at early phase of injury enhanced repair from ischemic brain injury which is associated with increased vessel regeneration.
Acknowledgements : This work was supported by the future based technology development program of the National Research Foundation (NRF) (2010-0020405) given to Prof. Youngsook Son.

F-3237

DEFINING THE SURFACE MOLECULAR SIGNATURE OF GROWTH AND DIFFERENTIATION IN THE HUMAN NEURAL LINEAGE

Gizem Turaç[1], Marcus Horl[1], Christopher Hindley[1], Jason Davis[1], Michela Deleidi[2], Erdal Karaöz[3], **Jan Pruszak**[1]

[1]Institute of Anatomy and Cell Biology, Dept. Molecular Embryology, University of Freiburg, Freiburg, Germany, [2]Hertie-Institute for Clinical Brain Research, German Center for Neurodegenerative Diseases (DZNE), University of Tübingen, Tübingen, Germany, [3]Center for Stem Cell and Gene Therapies Research and Practice, Kocaeli University, Kocaeli, Turkey

Surface molecule profiles can serve as markers of neural stem cell patterning and differentiation, are frequently altered in pathological processes and can be exploited for stem cell selection strategies and diagnostics. We report a comprehensive characterization of cluster of differentiation (CD) antigens during neural lineage differentiation of human pluripotent stem cells, cancer lines and primary cultures. Combinatorial surface antigen expression analysis uncovered a multitude of previously undefined neural cell populations. Functionally, the dynamics of chemokine receptor-4 (CXCR4, CD184) expression suggest its involvement in neural stemness and migration. Expression patterns of integrin heterodimers (CD29, CD49f) underline their role in niche adherence of human neural stem cells. Finally, CD49f and CD200 (OX2) combinatorial surface expression was found to define proliferative human neural stem cells versus differentiated neurons and enabled the isolation of neurons for future biomedical applications.

F-3238

AGING OF THE MOUSE SUBVENTRICULAR ZONE DOES NOT AFFECT THE ABILITY OF STEM CELLS TO PRODUCE NEUROBLASTS

Maria Apostolopoulou[1], Susan Goderie[1], Steve Lotz[1], Mark Winter[2], Eric Wait[2], Meryl Lindsey[1], Ashlyn DiRisio[1], Andy Cohen[2], Sally Temple[1]

[1]Neural Stem Cell Institute, Rensselaer, NY, United States, [2]Drexel University, Philadelphia, PA, United States

Stem cell systems decline with aging, and evidence points to both stem-cell-inherent as well as microenvironmental factors being critical to this decline. This study focuses on changes that occur in the stem cells themselves with age. To this end, we focused on the subventricular zone (SVZ), one of the two areas in the adult mammalian brain that retains neural stem cells, and the most active in terms of proliferation. SVZ stem cells (type B cells) are slowly proliferating cells. They can give rise, through asymmetric or symmetric division, to transit-amplifying cells (type C cells), which have some self-renewing capacity and exhibit high proliferation rate. Type C cells can in turn give rise to neuroblasts (type A cells), which may continue to proliferate during their migration to the olfactory bulb, where they give rise to interneurons. It is believed that type B cells can also give rise to oligodendrocytes. In our work we used live, time-lapse imaging to record the behavior of individual dissociated SVZ cells in vitro, and immunocytochemistry to determine the fate of their progeny. We observed a decline in the clonal capacity of aged SVZ cells. When comparing the fates of SVZ cell progeny, we observed no significant difference in the output of specific cell types other than a decrease in the numbers of oligodendrocytes produced with advanced age. We also observed a general lengthening of the cell cycle of aged cells. Our results suggest that the number of cells able to proliferate is depleted from the SVZ during aging, but the potential of these cells to produce neurons remains unchanged. These results are in agreement with previous reports of dwindling proliferating cell numbers in aged SVZ tissue. Our results also provide credence to potential therapies which use aged-patient-derived stem cells to produce beneficial cell types, by showing that these cells are comparable to ones derived from younger donors.

F-3241

DIFFERENTIAL VULNERABILITY OF ADULT NEUROGENESIS BY ADULT AND PRENATAL INFLAMMATION: ROLE OF TGF-BETA1

VALERIA ROCA, Mariana Graciarena, Patricia Mathieu, Fernando J. Pitossi
Leloir Institute, Buenos Aires, Argentina

Peripheral inflammation impairs adult neurogenesis,
both during the prenatal period and in the adulthood. We hypothesized that, similar

to other programming effects of prenatal treatments, only prenatal inflammation causes long-term consequences in adult neurogenesis and its neurogenic niche. To test this, pregnant Wistar rats were subcutaneously injected with lipopolyssaccharide (LPS; 0.5mg/kg) or saline solution every other day from gestational/embryonic day (GD) 14 to 20. In addition adult animals were injected with a single intraperitoneal saline or LPS injection (1mg/kg) and the effects on neurogenesis were assessed 7 days later. Alternatively, to evaluate long-term consequences of adult LPS injections, LPS (1mg/kg) was administered peripherally to adult rats 4 times every other day, and the effects on neurogenesis were assessed 60 days later.
Prenatal and adult LPS treatments reduced adult neurogenesis and provoke specific microglial (but not astroglial) activation in the DG. However, only prenatal inflammation-mediated effects were long-lasting (at least 60 days).Moreover, these effects were specific to the DG since the SVZ was not affected. In addition, these stimuli caused differential effects on the molecular components of the neurogenic niche; only prenatal LPS treatment reduced the local levels of TGF-β1 mRNA in the DG. In parallel, prenatal LPS also altered an adult neurogenesis-dependent behavioural task (Novel Object Recognition (NOR). When the reduced levels of TGF- β1 caused by prenatal LPS were increased by the adult administration of adenoviral vectors expressing this cytokine, adult neurogenesis levels and the performance in the NOR test were restored. Finally, TGF-β1 exerted its pro-neurogenic effects via the Smad2/3 pathway in a neural stem cell culture.
Taken together, these data add evidence to the duration, regional specificity and dramatic consequences of prenatal immune programming on CNS physiology, compared with the limited response observed in the adult brain and highlight the role of TGF-β1 on adult neurogenesis and NOR.

F-3242

THE MOOD STABILIZER VALPROATE ACTIVATES HUMAN FGF1 GENE PROMOTER THROUGH INHIBITING HDAC ACTIVITIES.

Chien Yu Kao[1], Yi Chao Hsu[2], Ing Ming Chiu[1]
[1]National Health Research Institutes, Miaoli, Taiwan, [2]Mackay Medical College, Sanzhi, Taiwan

Valproic acid (VPA) is the primary mood-stabilizing drug to exert neuroprotective effects and to treat bipolar disorder in clinic. Fibroblast growth factor 1 (FGF1) has been shown to regulate cell proliferation, cell division and neurogenesis. Human FGF1 gene 1B promoter (-540 to +31)-driven green fluorescence (F1BGFP) has been shown to recapitulate endogenous FGF1 gene expression and facilitates the isolation of neural stem/progenitor cells (NSPCs) from developing and adult mouse brains. In this study, we provide several lines of evidence to demonstrate the underlying mechanisms of VPA in activating FGF-1B promoter activity: (i) VPA significantly increased the FGF-1B mRNA expression and the percentage of F1BGFP(+) cells in human glioblastoma U-1240 MG/F1BGFP cells, mouse embryonic stem cells and F1BGFP transgenic mouse brain stem cells; (ii) the increase of F1BGFP expression by VPA involves changes of RFX1-3 transcriptional complexes on the 18-bp cis-element of FGF-1B promoter; (iii) treatments of other HDAC inhibitors, sodium butyrate and trichostatin A, significantly increased the expression levels of FGF-1B, RFX2 and RFX3 transcripts; (iv) treatments of GSK-3 inhibitor, lithium, or GSK-3 siRNAs also significantly activated FGF-1B promoter; (v) VPA and sodium butyrate specifically enhanced neuronal differentiation in F1BGFP(+) NSPCs rather than GFP(-) cells. This study suggested, for the first time, that VPA activates human FGF1 gene promoter through inhibiting HDAC activities.

F-3243

INVESTIGATION OF NEUROGENESIS IN A HUMAN XLID MOUSE MODEL

Chun-Yu Chen, Yu I-Shing, Hsu Yu-Chen, Hung Chia-Lun, Kao Chung-Yang, Lin Chien-Yu, Tsai Ming-Shian, Lin Shu-Wha
Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University, Taipei, Taiwan

Deletion of Cul4b, an X-linked gene which encodes a scaffold protein of the E3 ubiquitin ligase complex and is responsible for X-linked intellectual disability (XLID), in early cleavage stage in mice may cause early embryo lethality. Nevertheless, we successfully rescued the lethality of Cul4b knockout mice (Cul4bΔ/Y) by deleting Cul4b in epiblast stage using the Sox2-Cre transgenic mice expressing the Cre recombinase in epiblast stage and neuronal cell lineage. Our previous study has suggested that the Sox2-Cre; Cul4b deficient mice were a suitable mouse model of human XLID. In order to characterize the neuronal phenotype of the Sox2-Cre; Cul4b deletion mice, we studied the structure and organization of the cortex, striatum, and hippocampus in Cul4bΔ/Y mice and found no differences between Cul4bΔ/Y mice and WT littermate in these aspects except that the mice displayed spatial learning deficit in Morris water maze test. Spatial learning deficit is associated with impaired adult neurogenesis. We thus study the neurogenesis ability of the Cul4bΔ/Y mice. Immunostaining and quantification of proliferating neuron precursors were performed and the results showed that the early precursors markers, PSA-NCAM and calretinin, did not display significant differences between WT and Cul4bΔ/Y mice. The amount of Ki67 and BrdU immunoreactive cells in Cul4bΔ/Y mice also showed similar level to that in WT mice. Taken together these results indicated that the spatial learning deficit of Cul4bΔ/Y mice could not be explained by abnormalities in neurogenesis.

F-3244

NEURAL PROGENITOR CELL (NPC) ACTIVITY IN THE SUBVENTRICULAR ZONE (SVZ) AND SPINAL CORD DURING AMYOTROPHIC LATERAL SCLEROSIS (ALS) DISEASE PROGRESSION

Seth H. Cassel, Kevin C. Eggan
Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, United States

In order to assess neural progenitor cell (NPC) activity in amyotrophic lateral sclerosis (ALS) disease conditions, we bred nestin-CreER; lox-stop-lox mCherry; SOD1-G93A mice. We then tracked the labeled cells at various time points during disease progression. No increase in activity was noted in the SVZ. Additionally, neurogenesis in the olfactory bulb (OB) was not impacted. In the spinal cord, NPCs did not display altered behavior in most mice; however, in a subset of ALS model mice, NPC proliferation in the spinal cord was apparent. We suspect that the niche of these proliferating progenitors is in the parenchyma of the spinal cord. We conclude that the extent of cell death in ALS fails to trigger a widespread response from neural progenitor cells. Conversely, the presence of the SOD1-G93A transgene does not appear to impact the viability of the NPCs or their ability to form new neurons in the olfactory bulb.

F-3245

EXPLORING NON-CELL-AUTONOMY IN AN IPSC-DERIVED ASTROCYTIC AND NEURONAL MODEL OF ALZHEIMER'S DISEASE

Lauren K. Fong, Beatriz C.G. Freitas, Alysson R. Muotri, Lawrence S.B. Goldstein
University of California, San Diego, San Diego, CA, United States

Understanding the mechanism of Alzheimer's disease (AD) pathogenesis has been made difficult by the inability of animal models to fully recapitulate AD's molecular and cellular phenotypes. Recent human induced pluripotent stem cell (hiPSC)-derived neuronal models have shown it is possible to recapitulate the complex genetic diversity of a patient and more completely model disease initiation and progression. However, AD is a multifaceted disease that involves multiple cell types. To examine cell-autonomous and non-cell-autonomous mechanisms of AD patho-

genesis, we must expand our model to include both hiPSC-derived neurons and glia since it is likely that these cells are differentially affected by the disease process. Astrocytes, the most abundant non-neuronal cell type in the brain, have been implicated in AD in the clearance of the amyloid-β peptide and also in neuroinflammation. Using a new in vitro differentiation protocol to generate astrocytes from hiPSC, we have generated astrocytes that are positive for astrocyte markers: vimentin, GFAP, and CD44; for the glial progenitor marker, A2B5; for neural stem cell marker, Nestin; and negative for microglial marker, Iba1. We find that these hiPSC-derived astrocytes exhibit a distinct CD44-GFAP signature compared to neural precursor cells and neurons. Additionally, the relatively high yield of CD44+ cells from this differentiation protocol permits isolation of a CD44+ astrocyte-restricted population to a high degree of purity. Functional assays demonstrate that these hiPSC-derived astrocytes can internalize the major excitatory neurotransmitter, glutamate. Furthermore, they exhibit activation in response to LPS-stimulation, a glial response common in neurodegenerative diseases. This hiPSC-derived astrocytic-neuronal model could bring new insight to whether non-neuronal cells like astrocytes modulate AD neuronal phenotypes and also allow for exploration of the functional role of different APOE alleles in modulating astrocytic and neuronal phenotypes in AD.

F-3246

GENETICALLY ENGINEERED HUMAN PLURIPOTENT STEM CELL MODELS OF RETT SYNDROME

Yun Li[1], Haoyi Wang[1], Julien Muffat[1], Albert W. Cheng[1], David A. Orlando[1], Show-ming Kwok[2], Danielle A. Feldman3[2], Helen S. Bateup[3], Maisam Mitalipova[1], Bernardo L. Sabatini[3], Richard A. Young[1], Mriganka Sur[2], Rudolf Jaenisch[1]

[1]Whitehead Institute for Biomedical Research, MIT, Cambridge, MA, United States, [2]The Picower Institute for learning and memory, Massachusetts Institute of Technology, Cambridge, MA, United States, [3]Howard Hughes Medical Institute, Department of Neurobiology, Harvard Medical School, Boston, MA, United States

The advent of human pluripotent stem cell technology, including that of embryonic stem cells and induced pluripotent stem cells, has opened up a new avenue in human disease modeling. A crucial limitation in using these patient-specific pluripotent stem cells is the lack of genetically-matched controls. Here we report the generation of isogenic human pluripotent stem cell model of Rett Syndrome by genetically modifying the MECP2 gene. Using TALEN-mediated gene targeting, we have introduced loss-of-function mutation into the MECP2 gene, and generated hemizygous, heterozygous and homozygous null cells. These isogenic human pluripotent stem cells were differentiated into neural progenitor cells as well as mature neurons and astrocytes. We showed that neurons derived from MECP2 null pluripotent stem cells have smaller soma and reduced neurite arborization compared to their isogenic controls. Further analyses revealed that MECP2 null neurons bare key molecular, cellular and physiological features of Rett Syndrome. Using an unbiased global gene expression analysis approach, we uncovered a pattern of genome-wide reduction of expression in MECP2 null neurons. In summary, our results demonstrate the feasibility TALEN-mediated gene targeting in generating an in vitro model of human neurodevelopmental disorder. By dissecting disease-related phenotypes in MECP2 null neurons, we hope to investigate disease mechanism, and develop therapeutic strategies that ameliorate these phenotypes in vitro and in vivo.

F-3247

NEURONAL DIFFERENTIATION OF AUTISM SPECTRUM DISORDER SPECIFIC FIBROBLAST DERIVED iPSCs TO FUNCTIONAL NEURONS AND GLIA

David J. Brick[1], Hubert E. Nethercott[1], Maria G. Banuelos[1], Alexander E. Stover[1], Samantha Montesano[1], Randi J. Hagerman[2], Michele Ono[2], David Hessl[2], Lei Sun[3], Diane K. O'Dowd[3], Philip H. Schwartz[1]

[1]Centers for Neuroscience and Translational Research, CHOC Children's Research Institute, Orange, CA, United States, [2]Pediatrics, UC Davis MIND Institute, Sacramento, CA, United States, [3]Developmental and Cell Biology, UC Irvine, Irvine, CA, United States

Autism Spectrum Disorders (ASDs) are the fastest growing developmental disorder in the United States and are, unfortunately, still poorly understood. A critical barrier in autism research is limited availability of statistically relevant numbers of patient-derived tissue samples, especially those of neural origin. Recent advances in the field of stem cell biology now make it possible to derive induced pluripotent stem cells (iPSCs) from patient-specific ASD cell lines. These can be used for a wide variety of studies from cellular analysis of disease mechanisms to screening for new therapeutics. Access to ASD patient cells lines, however, is still a limiting factor in the field. Here, we describe a newly established ASDs fibroblast and iPSC cell line resource. We currently have over 100 lines, available for request, derived from healthy and clinically-well-defined ASD-specific male patients and unaffected volunteers. A challenge in using iPSC disease models is to efficiently produce relevant differentiated and functional cell types for analysis. Using methods developed in our laboratory, we have confirmed, using a subset of the normal and ASD-specific iPSCs, that these cells can be differentiated toward a neural stem cell phenotype and terminally differentiated into action-potential firing neurons as well as glia. This represents a significant resource that will advance the use of ASD patient cells as disease models by the scientific community. A comparative analysis of these ASD-specific lines with unaffected controls will facilitate greater insight into the cause(s) and biology of the ASDs.

F-3248

BASAL RADIAL GLIA IN OUTER SUBVENTRICULAR ZONE MAY CONTRIBUTE TO GYRUS AND SULCUS FORMATION IN THE DEVELOPING MARMOSET NEOCORTEX

AY Murayama, Hideyuki Okano
Keio Univ Sch of Medicine, Tokyo, Japan

Recent studies revealed a novel type of subventricular zone (SVZ) progenitor, referred to as basal radial glia (bRG), which retains a basal process to the pial surface, sustains expression of radial glial markers and is capable of self-renewal. bRGs occur at high relative abundance in the outer SVZ (OSVZ) of gyrencephalic animals, but lower of lissencephalic rodents, suggesting that the division of bRG in OSVZ is important to generate gyrencephalic brain. The common marmoset, Callithrix jacchus, is a primate which has fewer gyrus and sulcus formation but the abundance of bRG cells in its OSVZ is similar to that in human.
In this study, we characterized bRG cells using the technique of sequential BrdU and EdU labeling by intraperitoneal injection to pregnant mother, in combination with immunofluorescent staining of brain slice. In addition, we performed live imaging of bRG using a cortical slice culture technique and observed the birth of bRG cells, their division pattern in OSVZ and the fate of their daughter cells. We found that the cell cycle length of bRG of marmoset may be longer than that of human.
Furthermore, by histological analysis, the OSVZ in the presumptive sulcal region at E92 (embryonic day 92) was found to be thinner than that in non-sulcal regions. The result suggested that the bRG in OSVZ contributes to the girus-sulcal formation in the developing marmoset cerebral cortex

F-3251

THE EPIGENETIC REGULATOR BRD2 CONTROLS POSTNATAL HIPPOCAMPAL NEUROGENESIS

Woosung Cho[1], Sangkyun Lim[1], Tracey Shipman[1], Efrain Sanchez-Ortiz[1], Wei Mo[1], Anahita Amiri[2], Renee McKay[1], Luis Parada[1]
[1]Developmental Biology, UT-Southwestern Medical Center, Dallas, TX, United States, [2]Neurobiology, Yale University, New Haven, CT, United States

Neural stem cells (NSCs) are the source of lifelong generation of new neurons in the mammalian brain. Various molecular mechanisms that are spatiotemporally regulated contribute to the neurogenic process in the postnatal brain. Impaired postnatal hippocampal neurogenesis has been implicated in many neurological and psychiatric disorders, including Parkinson's disease and depression. Bromodomain proteins (BRDs) contain bromodomains that have a strong affinity to hyper-acetylated histone residues and regulate their target genes by recruiting additional transcriptional factors. Thus, they translate the epigenetic codes of histone acetylation into gene regulation. Brd2

is a member of BRDs that are highly expressed in the developing central nervous system, and has been identified as the major susceptibility gene for human *Juvenile Myoclonic Epilepsy.* Here we show that Brd2 knockdown in cultured murine postnatal NSCs and in the mouse hippocampus suppresses neuronal differentiation. Hypomorphic Brd2 NSCs exhibited a decreased number of newborn neurons marked by doublecortin and NeuroD1 when differentiation was induced *in vitro*. However, glial differentiation was unaffected. Brd2 knockdown also led to delayed neuronal maturation and slowed the migration of newborn neurons from the sub-granular layer to the granule layer of the dentate gyrus *in vivo*. Brd2 controls histone acetylation-mediated activation of the proneural bHLH genes. Brd2 knockdown caused no change in histone acetylation status of the NSCs. However, hypomorphic Brd2 abrogated upregulation of NeuroDs and neurogenins mediated by *Trichostatin A, a potent HDAC inhibitor* that induces global histone hyperacetylation. Overexpression of Brd2 accelerated NSC proliferation accompanied with increased levels of E2F1 and cyclin A. These results emphasize the requirement of Brd2 in postnatal hippocampal neurogenesis. Notably, we demonstrate that translation of the histone modification code provides a new level of epigenetic regulation for fine-tuning gene regulation.

F-3252

COGNITIVE PERFORMANCE IN MICE UPON IN VIVO EXPANSION OF NEURAL STEM CELLS AND NEUROGENESIS

zhongwei sun, Alexander Garthe, Gerd Kempermann, Federico Calegari
CRTD / DFG-Center for Regenerative Therapies Dresden - Cluster of Excellence, dresden, Germany

Despite many progresses in recent years, the function of adult mammalian neurogenesis is still debated. Our laboratory has recently found that a transitory overexpression of cdk4/cyclinD1 by stereotaxic viral injection in the hippocampus can be used to increase the expansion of neural stem cells of adult mice in order to increase neurogenesis (Artegiani et al., 2011). Using this system, we applied a number of paradigms to assess hippocampal function including the assesment of spatial learning, re-learning and memory performance. Data suggesting improved cognitive performace will be presented.

F-3253

MARKED EXPRESSION OF MALE SPECIFIC GENES DURING DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO DOPAMINERGIC NEURONS

Mehdi sharifi tabar[1], Ali fathi[2], Haghighat vakilian[1], mehdi alikhani[1], Hossein baharvand[1], Ghasem hosseini salekdeh[1]
[A]Systems biology, [B]Stem Cells and Developmental Biology Group of Cell Science Research Center,, [1]Royan, tehran, Iran, Islamic Republic of

Marked expression of male specific genes during differentiation of human embryonic stem cells into dopaminergic neurons

Mehdi Sharifi Tabar[1], Ali Fathi[2], Haghighat vakilian[1], Mehdi Alikhani[1], Hossein Baharvand[2], Ghasem Hosseini Salekdeh[1*]

[1 *]Molecular Systems Biology Group of Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

[2] Stem Cells and Developmental Biology Group of Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

The genetic basis of sex differences in the neuron development is quite challenging. Omics and epidemiological evidences suggest that the male gender is one of the risk factors for the development of Parkinson disease (PD). However, molecular evidences in gender-biased neurological disorder are still lacking. Here we used human embryonic stem cells (hESCs) as a model for recapitulating mesencephalon midbrain dopaminergic neurons development, and expression of gender specific genes were analyzed at 5, 11, 15 days after neural differentiation. Differentiated cells were characterized for expression of specific dopaminergic markers such as LMX1a, LMX1b, PITX3, TH

and NURR1 using qRT-PCR and Immunofluorescence staining. We then profiled the expression of 21 Y chromosome genes and nine of their X-homologues during ESC differentiation to dopaminergic cells. Eleven days after differentiation initiation, we observed a significant increase (more than 50 folds) for DDX3Y, RBMY1, PCDH11Y, BPY2, CDY1, and HSFY1 transcripts and proteins compared to their X- homologues. These data suggest that male specific genes, which are well known for their important role in spermatogenesis, could function in neurogenesis as well, and guarantee further investigation for functional analysis of male specific genes in dopaminergic neuron development in human.

Key Words: Embryonic Stem Cells, Dopaminergic Neurons, Y chromosome, Real-Time PCR, Western Bloting

F-3254

EXPRESSION AND FUNCTIONAL STUDY OF RUFY3 DURING MOUSE ESC NEURAL DIFFERENTIATION AND MOUSE NEOCORTEX DEVELOPMENT

Ming Sun, Zhe Wei, Ying Jin
Institute of Health Sciences, ShangHai, China

In order to screen novel regulators involved in neural differentiation from mouse embryonic stem cells (ESCs), we analyzed previous published microarray data by others and our laboratory. We identified several candidate genes, which were up regulated during mouse ESC neural differentiation and had not been well characterized functionally during this process. Among the candidates, Rufy3 (RUN and FYVE domain containing 3) was chosen for further study. Rufy3 was significantly up regulated in our modified SFEB (serum-free embryoid body) neural differentiation model at both mRNA and protein levels. Immunostaining results indicated that Rufy3 was exclusively expressed in ESC-derived neurons but not in either ESC-derived neural progenitor cells or glial cells. Similarly, Rufy3 was exclusively detected in neurons when primary mouse cortical neural cells were examined. Moreover, throughout the process of neocortical development, Rufy3 was highly and specifically expressed in the multi-layers of neocortex. Thus, Rufy3 could be a new marker for the neuron. Functionally, over expression of Rufy3 in cortical neurons gave rise to longer axons, whereas the numbers of total branches and axons were not changed. In contrast, knock down of Rufy3 reduced the length of axon and the number of branches. Neurons with no axons were also found in certain fraction of neurons after the silencing of Rufy3 in cultured cortical neuron. However, results of in utero electroporation (IUE) showed that over expression or knock down of Rufy3 had no obvious effect on the migration of neural cells in vivo. Mechanistically, we found that Rufy3 was expressed in vesicular like structure in primary cortical neurons and partially colocalized with Rab5 and Rab9,markers for early and late endosome, respectively. These results suggest that Rufy3 might be involved in the intracellular vesicle trafficking and regulate neuron axonal outgrowth. Recently, we have generated the conditional Rufy3 knock out mouse, which will greatly facilitate our investigation of the expression and function of Rufy3 systematically.

F-3255

GRAFTING OF ENTERIC NEURAL STEM/PROGENITOR CELLS INTO THE BRAIN AFTER RADIOTHERAPY

Ahmed Osman, Klas Blomgren
1.Department of Women's and Children's Health, Karolinska Institute, Stockholm, Sweden

Radiotherapy is an essential and efficient treatment tool for brain tumors. However, cranial irradiation (IR) often results in cognitive deficits in pediatric cancer survivors.
To date, neurogenesis is widely accepted to occur throughout life in two discrete regions in the brain, the subventricular zone and the dentate gyrus of the hippocampus, the latter of which has been linked to learning and memory. Due to their high proliferative capacity, neural stem cells are vulnerable to ionizing radiation, and IR-induced depletion of neural stem cells appears to be long-lasting, even after a single, moderate dose of IR. Cell therapy has been shown to ameliorate the cognitive deficits caused by IR in rodents. However, such an approach requires a clinically relevant source of neural stem cells. Here we hypothesized the enteric neural stem/progenitor

cells (ENSPC), which develop from the neural crest during embryogenesis and exist in the intestinal wall, as candidate autologous cell type.
Cells were isolated from the intestinal wall and propagated *in vitro*
for one week. Differentiation assays showed that ENSPC are multipotent and generate neurons, astrocytes and myofibroblasts.
To investigate if ENSPC can be used *in vivo*, the brains of postnatal day 9
(P9) female mice were subjected to a single irradiation dose of 8 Gy. Twelve days later, mice (sham or IR) received an intrahippocampal
injection of CM-DiI-labeled ENSPC (left hemisphere) or vehicle (right
hemisphere). Four weeks after grafting, 0.5% and 1% of the grafted cells were detected in the dentate gyrus of the sham and IR animals, respectively.
It remains necessary to characterize the grafted cells, to assess morphological effects on the brain and to evaluate cognitive functions after ENSPC transplantation.

F-3256

UNCOVERING FATE POTENTIAL, TRANSCRIPTOMIC AND EPIGENOMIC FOUNDATIONS OF HUMAN NEURAL STEM CELL ONTOGENY USING TRANSGENIC REPORTER HUMAN ES CELLS FOR NOTCH SIGNALING

Reuven Edri[1], Micahel J. Ziller[2], Alon Goren[2], Yakey Yaffe[1], Anna Dukhovny[1], Bradley E. Bernstein[2], Alexander Meissner[2], Yechiel Elkabetz[1]
[1]Cell and Developmental Biology, Tel Aviv University, Tel Aviv, Israel, [2]Broad Institute, Cambridge, MA, United States

Neural stem cells (NSCs) exhibit dynamic heterogeneity with respect to their self-renewal and cell fate potential properties both in vivo and in vitro, which is well reflected in their poor ability to yield various neuronal cell types. We are aimed at developing strategies to convert human pluripotent stem cells (PSCs) into distinct NSC types, which in analogy to self-renewing PSCs, should provide unlimited neural cell sources for a spectrum of neuronal and glial fates.
We established a long-term neural differentiation culture using a Hes5::GFP BAC transgenic human embryonic stem cell (hESC) line, which reliably marks neural stem / progenitor cells active for Notch signaling throughout neural development. Using this in vitro paradigm we recapped the neuronal to glial cell fate progression during neural development, and identified distinct NSC stages based on their Notch activation state.
Hes5+ populations exhibited a strong neural stem/progenitor cell state signature throughout the entire culture period, as reflected by expression of general NSC markers (Nestin, Sox2, Pax6). Nonetheless, strikingly distinct stage specific molecular signatures and functional properties were observed among the various Hes5+ populations as well as between Hes5+ and Hes5- cells at each stage. These data allowed for the first time a reliable recapitulation of lineage related neural stem / progenitor cell stages: neuroepithelial cells, neurogenic radial glial cells, gliogenic radial glial cells and adult-like NSCs.
Cell sorting, fate potential assays and two-photon live cell imaging show that neural progenitors firstly emerging from PSCs segregate in a 2-way switch manner into Hes5+ neuroepithelial cells and Hes5- neural crest and placode cell fates. Hes5+ neuroepithelial cells give rise to rosette-neural stem cells (R-NSCs) - highly polarized columnar cells with both epithelial and astroglial characteristics - corresponding to early radial glia cells. Early Hes5+ but not Hes5- cells exclusively responded to early developmental cues and could differentiate into region specific fates such as spinal cord motoneurons, but could also be directed to early cortical plate cell fates. In contrast, Hes5+ R-NSCs exhibited VZ-like neurogenic radial glial identity and yielded deep layer corticospinal neurons, while later R-NSC-derived progenitors exhibited SVZ-like Tbr2+ basal progenitors and yielded upper layer cortical neurons. Hes5+ radial glial cells continued to self renew in culture, developing into late embryonic SVZ-like progenitors expressing EGFR with strong gliogenic potential. Long-term cultured R-NSCs largely lacked Hes5, responded to FGF2/EGF and contained tri-potent NSCs yielding olfactory bulb neurons and glia. Hes5+ and Hes5- NSCs from these stages were grafted in rat host brains and are now processed for cell fates.

Extensive epigenetic analysis of all Hes5+ expressing cells including whole genome DNA methylation, 5 histone mark modifications, RNA-Seq and miRNA profiling, revealed for the first time a comprehensive epigenetic road map for human NSC ontogeny. These data demonstrate a clear progressive, irreversible hypermethylation in key developmental genes along the entire differentiation process, further manifesting the critical timing consideration in cell fate specification in vitro. Using computational approaches we are developing strategies for direct epigenetic reprogramming towards distinct NSC states and specific neuronal and glial fates.