

SEPT

15-18



STEM CELLS
IN TRANSLATION
FLORENCE, ITALY

FINAL PROGRAM

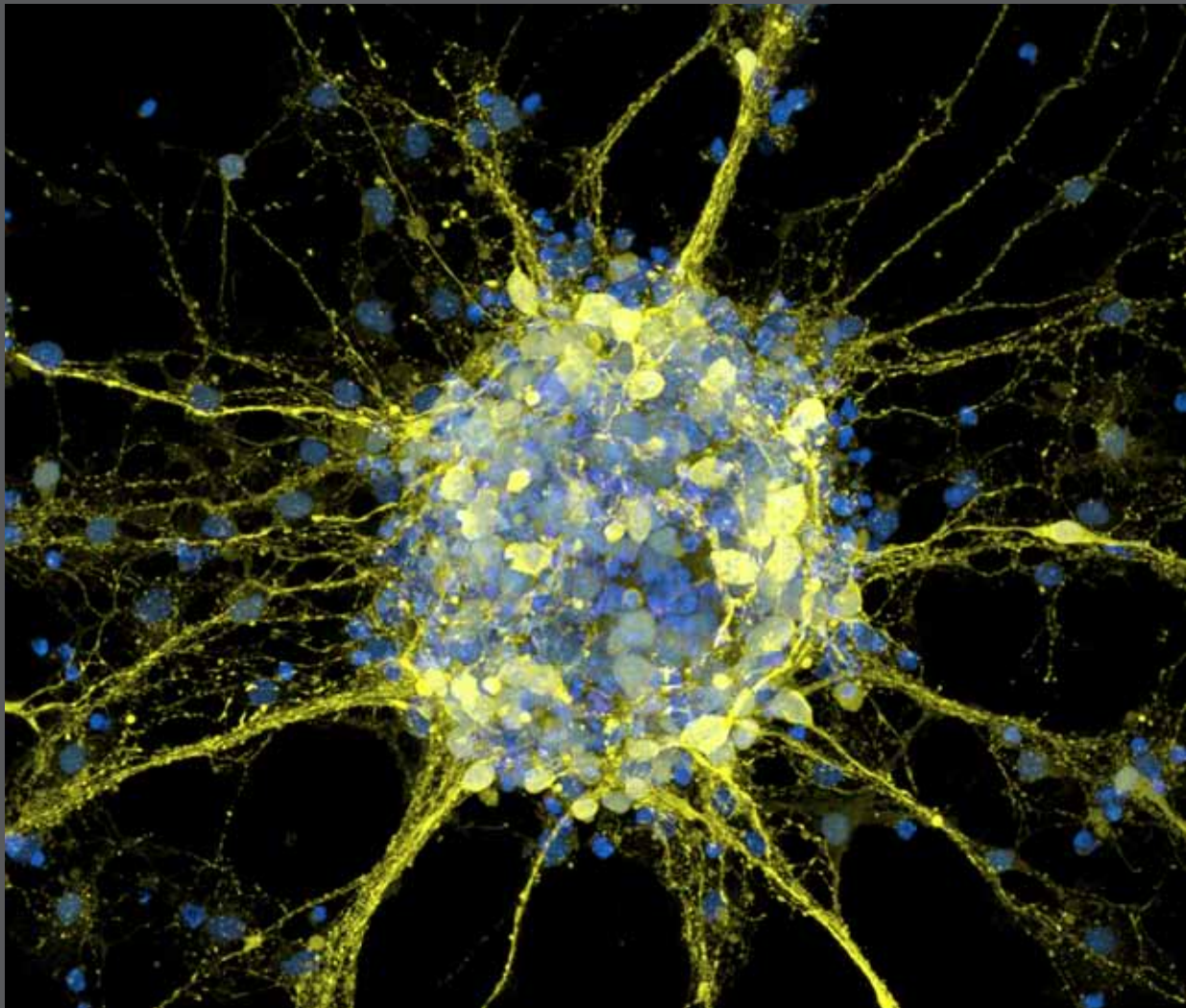
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FEATURED SPEAKERS:

Connie J. Eaves, PhD

Terry Fox Lab BC Cancer Agency, Canada

Brigid L. Hogan, PhD

Duke University Medical Center, USA

Gordon M. Keller, PhD

McEwen Centre for Regenerative Medicine, Canada

Robin Lovell-Badge, PhD, FRS

MRC National Institute for Medical Research, UK

Olivier Pourquie, PhD

IGBMC, France

Lorenz Studer, MD

Sloan-Kettering Institute for Cancer Research, USA

REGISTRATION AND ABSTRACTS OPEN 12/12/13
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Dear colleagues,

On behalf of the International Society for Stem Cell Research, we are delighted to welcome you to Florence, the cradle of the Renaissance, for our Regional Forum “Stem Cells in Translation.” Here, you’ll have an unprecedented opportunity to learn about breakthroughs being made at the frontier of discovery as researchers seek to deliver the incredible promise of stem cell research to patients in a clinical setting.

Our goal, like that of the artists, philosophers and scientists who studied in salons throughout this historic city, is to nurture a confluence of passion, information and curiosity that stimulates thought and propels advances in our dynamic, challenging field.

Hear from a slate of 37 distinguished speakers including clinician scientists leading ground-breaking clinical trials on cell therapy, gene therapy, cancer treatment and more. Keynote Speaker John Dick will discuss “Stem Cells in Cancer: Do They Matter?” and Closing Keynote Speaker Gordon Keller will address “Directed Differentiation of Human Pluripotent Stem Cells.”

Survey more than 150 posters that provide a wide-ranging view of the latest findings and scientific advances in translational research.

And finally, enjoy the Forum’s relaxed, collegial setting designed to help you make the most of your access to many of your most prominent, knowledgeable peers. You will have ample time to ask questions, exchange information, share ideas and network, either at poster presentations or during our receptions.

And, of course, experience all this in historic Florence, a city whose beauty and culture has inspired leading thinkers for centuries.

As always, we are grateful for your support and participation. We believe you’ll find this rare convergence of prominent translational researchers and cutting-edge translational science stimulating, thought-provoking and memorable.



Cedric Blanpain,
Université Libre de Bruxelles, Belgium



Michele De Luca,
University of Modena and Reggio Emilia, Italy



Ronald McKay,
Lieber Institute for Brain Development, USA



Richard Young,
Whitehead Institute for Biomedical Research, USA

Organizing Committee
ISSCR Regional Forum, Florence 2013



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PROGRAM SCHEDULE

SUNDAY, SEPTEMBER 15

2:00-9:00 PM REGISTRATION OPEN

7:00-9:00 PM OPENING SESSION

7:00-7:15 **Opening Remarks: Michele De Luca**,
University of Modena and Reggio Emilia, Italy

7:15-8:30 **Keynote: John Dick**, *Campbell Family Cancer Research Institute, Ontario Cancer Institute, Princess Margaret Hospital, University Health Network, Canada*
STEM CELLS IN CANCER: DO THEY MATTER?

8:30-10:00 PM OPENING RECEPTION

MONDAY, SEPTEMBER 16

8:15-9:00 AM MORNING COFFEE BREAK

9:00 AM-12:10 PM **SESSION II: TISSUE HOMEOSTASIS**
Chair: Sally Temple, *Neural Stem Cell Institute, USA*

9:00-9:10 **Nancy Witty**, *ISSCR*

9:10-9:15 **Chair overview**

9:15-9:40 **Hans Clevers**, *Hubrecht Institute, Netherlands*
WNT SIGNALING, LGR5 STEM CELLS AND CANCER

9:40-10:05 **Frank McKeon**, *Jackson Laboratory for Genomic Medicine, USA*
DISSECTING THE STEM CELL COMPONENT OF PRECANCEROUS LESIONS

10:05-10:30 **Elaine Fuchs**, *Howard Hughes Medical Institute, The Rockefeller University, USA*
SKIN STEM CELLS IN SILENCE, ACTION AND CANCER

10:30-10:55 **Refreshment Break**

10:55-11:20 **Ben Simons**, *University of Cambridge, UK*
MOUSE SPERMATOGENIC STEM CELL SELF-RENEWAL

11:20-11:45 **Sean Morrison**, *Children's Medical Research Institute at UT Southwestern, USA*
STEM CELLS: LOST IN TRANSLATION

11:45-12:10 **Holger Gerhardt**, *London Research Institute-Cancer Research UK, UK*
FROM CELLS TO NETWORKS-PRINCIPLES OF VASCULAR PATTERN FORMATION

12:10-2:00 PM **LUNCH BREAK, EXHIBITS AND POSTER VIEWING**

2:00-5:00 PM **SESSION III: CELL FATE CONTROL**
Chair: Richard Young, *Whitehead Institute for Biomedical Research, USA*

2:00-2:05 **Chair overview**

2:05-2:30 **Shahin Rafii**, *Cornell Medical College & Angiocrine Bioscience, USA*
CONTRIBUTION OF THE REPROGRAMMED VASCULAR NICHE TO STEM CELL SELF-RENEWAL AND ORGAN REGENERATION

2:30-2:55 **Markus Grompe**, *Oregon Health and Science University, USA*
THE ROLE OF EPITHELIAL PROGENITORS IN LIVER INJURY

2:55-3:20 **Deepak Srivastava**, *Gladstone Institute of Cardiovascular Disease and University of California San Francisco, USA*
REPROGRAMMING APPROACHES TO CARDIOVASCULAR DISEASE

3:20-3:45 **Refreshment Break**

3:45-4:10 **Lorenz Studer**, *Sloan-Kettering Institute for Cancer Research, USA*
HUMAN ES CELL DERIVED MIDBRAIN DOPAMINE NEURONS FOR CELL THERAPY IN PARKINSON'S DISEASE

4:10-4:35 **Robin Franklin**, *Wellcome Trust and MRC Cambridge Stem Cell Institute, UK*
TOWARDS REGENERATIVE MEDICINE FOR MULTIPLE SCLEROSIS

4:35-5:00 **Dieter Egli**, *New York Stem Cell Foundation, USA*
GENOME EXCHANGE IN HUMAN OOCYTES

5:00-5:30 **Sponsored by BD Biosciences: Mirko Corselli**, *Research and Development, BD Biosciences*
FLOW CYTOMETRY APPLICATIONS FOR ISOLATING AND ANALYZING COMPLEX HETEROGENEOUS STEM CELL CULTURES

5:30-7:00 **POSTER PRESENTATION AND RECEPTION**

TUESDAY, SEPTEMBER 17

8:15-9:00 AM MORNING COFFEE BREAK

9:00 AM-12:00 PM **SESSION IV: CELL THERAPY**

Chair: **Michele De Luca**, *University of Modena and Reggio Emilia, Italy*

9:00-9:05 **Chair overview**

9:05-9:30 **Yoshiki Sasai**, *Center for Developmental Biology RIKEN, Japan*
SELF-ORGANIZATION OF NEURAL STRUCTURES IN 3D STEM CELL CULTURE

9:30-9:55 **Graziella Pellegrini**, *University of Modena and Reggio Emilia, Italy*
REGENERATIVE MEDICINE FROM BENCH TO BESIDE: OVERCOMING THE CHALLENGES TO RESTORE VISION

9:55-10:20 **Paolo Macchiarini**, *ACTREM, Karolinska Institute, Sweden*
INTERACTION BETWEEN MESENCHYMAL STROMAL CELLS, BIOENGINEERED TISSUES AND ORGANS, AND HUMAN BODY

10:20-10:45 **Refreshment Break**

10:45-11:10 **Giulio Cossu**, *University College London, UK*
A PHASE I/II CELL THERAPY TRIAL FOR DUCHENNE MUSCULAR DYSTROPHY

11:10-11:35 **Frank Luyten**, *University Hospitals KU Leuven, Belgium*
CELL BASED THERAPEUTICS FOR SKELETAL APPLICATIONS

11:35-12:00 **Mauro Giacca**, *International Centre for Genetic Engineering and Biotechnology, Italy*
SECRETED FACTORS AND MICRORNAS INDUCING CARDIAC REGENERATION

12:00-2:00 PM **LUNCH BREAK, EXHIBITS AND POSTER VIEWING**

2:00-5:00 PM **SESSION V: GENE THERAPY**

Chair: **Henrik Semb**, *Lund University, Sweden*

2:00-2:05 **Chair overview**

2:05-2:30 **Bobby Gaspar**, *UCL Institute of Child Health, UK*
GENE THERAPY FOR SEVERE IMMUNODEFICIENCIES

2:30-2:55 **Luca Biasco**, *San Raffaele Scientific Institute, Italy*
UNCOVERING HEMATOPOIETIC STEM CELLS DYNAMICS AND ACTIVITY IN VIVO THROUGH VECTOR INSERTION SITE BARCODING IN HUMANS

2:55-3:20 **Alain Fischer**, *CHU Necker INSERM U768, France*
ADAPTIVE IMMUNODEFICIENCY AND GENE THERAPY

3:20-3:45 **Refreshment Break**

3:45-4:10 **Christopher Baum**, *Hannover Medical School, Germany*
PRECLINICAL GENOTOXICITY TESTING OF STEM CELL PRODUCTS

4:10-4:35 **Didier Trono**, *Federal Polytechnic School of Lausanne EPFL, Switzerland*
ENDOGENOUS RETROELEMENTS, KRAB'N'KAP, AND THE PRESERVATION OF STEM CELL TRANSCRIPTIONAL DYNAMICS

4:35-5:00 **Luigi Naldini**, *San Raffaele Scientific Institute, Italy*
GENETIC ENGINEERING OF HEMATOPIETIC STEM CELLS

WEDNESDAY, SEPTEMBER 18

8:15-9:00 AM MORNING COFFEE BREAK

9:00 AM-12:00 PM **SESSION VI: DEFINING**

REGENERATIVE MECHANISMS

Chair: **Ronald McKay**, *Lieber Institute for Brain Development, USA*

9:00-9:05 **Chair overview**

9:05-9:30 **Paolo Bianco**, *Sapienza University of Rome, Italy*
COMMERCIAL STEM CELLS - THE LESSON FROM THE MSC CASE

9:30- 9:55 **Shahragim Tajbakhsh**, *Institut Pasteur, Stem Cells & Development, France*
MOLECULAR REGULATION OF SKELETAL MUSCLE STEM CELLS

9:55-10:20 **David Sassoon**, *INSERM, University of Pierre and Marie Curie-Sorbonne University, France*
DISSECTING THE STEM CELL NICHE FOR THERAPEUTIC TARGETING

10:20-10:45 **Refreshment Break**

10:45-11:10 **Sally Temple**, *Neural Stem Cell Institute, USA*
DEVELOPING HUMAN RPE STEM CELLS FOR RETINAL REPAIR

11:10-11:35 **George Q. Daley**, *Boston Children's Hospital, Harvard Stem Cell Institute, Howard Hughes Medical Institute, USA*
PLURIPOTENT STEM CELLS IN DISEASE MODELING

11:35- 12:00 **Stuart Forbes**, *University of Edinburgh, UK*
THE CONTROL OF HEPATIC PROGENITOR
CELLS FOR LIVER REGENERATION

12:00-2:00 PM **LUNCH BREAK, EXHIBITS AND
POSTER VIEWING**

2:00-5:30 PM **SESSION VII: LINEAGE EPIGENETICS**
Chair: **Cedric Blanpain**, *Université Libre de
Bruxelles, Belgium*

2:00-2:05 **Chair overview**

2:05-2:30 **Tariq Enver**, *UCL Cancer Institute, University
College London, UK*
SYSTEMS LEVEL ANALYSIS
OF HEMATOPOIESIS

2:30-2:55 **Frederic de Sauvage**, *Genentech Inc., USA*
TARGETING DEVELOPMENTAL
PATHWAYS IN CANCER CELLS
AND STEM CELLS

2:55-3:20 **Salvador Aznar-Benitah**, *Institute for
Biomedical Research (IRB), Spain*
CHARACTERIZING CELL HETEROGENEITY
AND METASTASIS INITIATING EVENTS IN
HUMAN SCC

3:20-3:45 **Refreshment Break**

3:45-4:10 **John Stingl**, *Cancer Research UK, Cambridge
Research Institute, UK*
INTRATUMOURAL HETEROGENEITY IN
HUMAN SEROUS OVARIAN CARCINOMA

4:10-4:35 **Margaret Goodell**, *Baylor College of Medicine, USA*
REGULATION OF HEMATOPOIETIC
STEM CELLS BY DE NOVO DNA
METHYLTRANSFERASES

4:35-5:20 **Closing Keynote: Gordon Keller**, *McEwen
Centre for Regenerative Medicine/University
Health Network, Canada*
DIRECTED DIFFERENTIATION OF
HUMAN PLURIPOTENT STEM CELLS

5:20-5:30 **Closing Remarks: Michele De Luca and
Ronald McKay**



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SPEAKER ABSTRACTS

Sunday, September 15

OPENING SESSION 7:00 – 9:00 PM

Stem Cells in Cancer: Do They Matter?

John Dick

Campbell Family Cancer Research Institute, Ontario Cancer Institute, Princess Margaret Hospital, University Health Network, Canada

The cellular and molecular basis for intra-tumoral heterogeneity is poorly understood. Tumor cells can be genetically diverse due to mutations and clonal evolution resulting in intra-tumoral functional heterogeneity. Often proposed as mutually exclusive, cancer stem cell (CSC) models postulate that tumors are cellular hierarchies sustained by CSC heterogeneity due to epigenetic differences (i.e. long term tumor propagation only derives from CSC). Two lines of evidence support the CSC model in Acute Myeloid Leukemia (AML) and B-cell Acute Lymphoblastic Leukemia (B-ALL). We have recently developed gene signatures specific to either Leukemia Stem Cell (LSC) LSC or normal hematopoietic stem cells (HSC) and found they share a set of genes that define a common stemness program. Only these stem cell related gene signatures were significant independent predictors of patient survival in large clinical databases. Thus, determinants of stemness influence clinical outcome of AML establishing that LSC are clinically relevant and not artifacts of xenotransplantation. Second, we have carried out a series of combined genetic and functional studies of the Leukemia-initiating cells (L-IC) from either B-ALL or AML that point to commonalities between clonal evolution and CSC models of cancer. LSC from diagnostic patient samples were genetically diverse and reconstruction of their genetic ancestry showed that multiple LSC subclones were related through a complex branching evolutionary process. The discovery that specific genetic events influence L-IC frequency and that genetically distinct L-IC evolve through a complex evolutionary process indicates that genetic and functional heterogeneity are closely connected. Finally, we have also begun to study paired diagnostic (Dx) and relapse (Rx) samples and found that rare Dx subclones are chemotherapy resistant and become enriched at Rx. Our study points to the need to develop effective therapies to eradicate all genetic subclones to prevent further evolution and recurrence.

Monday, September 16

SESSION II 9:00 AM – 12:10 PM

Tissue Homeostasis

Wnt Signaling, Lgr5 Stem Cells and Cancer

Hans Clevers

Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences & University Medical Centre Utrecht, Netherlands

The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. We originally defined Lgr5 as a Wnt target gene, transcribed in colon cancer cells. Two knock-in alleles revealed exclusive expression of Lgr5 in cycling, columnar cells at the crypt base. Using an inducible Cre knock-in allele and the Rosa26-LacZ reporter strain, lineage tracing experiments were performed in adult mice. The Lgr5+ve crypt base columnar cells (CBC) generated all epithelial lineages throughout life, implying that it represents the stem cell of the small intestine and colon. Similar observations were made in hair follicles and stomach epithelium.

Single sorted Lgr5+ve stem cells can initiate ever-expanding crypt-villus organoids in 3D culture. Tracing experiments indicate that the Lgr5+ve stem cell hierarchy is maintained in these organoids. We conclude that intestinal crypt-villus units are self-organizing structures, which can be built from a single stem cell in the absence of a non-epithelial cellular niche. The same technology has now been developed for the Lgr5+ve stomach stem cells.

Intestinal cancer is initiated by Wnt pathway-activating mutations in genes such as APC. As in most cancers, the cell of origin has remained elusive. Deletion of APC in stem cells, but not in other crypt cells results in progressively growing neoplasia, identifying the stem cell as the cell-of-origin of adenomas. Moreover, a stem cell/progenitor cell hierarchy is maintained in early stem cell-derived adenomas, lending support to the "cancer stem cell"-concept.

Fate mapping of individual crypt stem cells using a multicolor Cre-reporter revealed that, as a population, Lgr5 stem cells persist life-long, yet crypts drift toward clonality within a period of 1-6 months. Lgr5 cell divisions occur symmetrically. The cellular dynamics are consistent with a model in which the resident stem cells double their numbers each day and stochastically adopt stem or TA fates after cell division. Lgr5 stem cells are interspersed between terminally differentiated Paneth cells that are known to produce bactericidal products. We find that Paneth cells are CD24+ and express EGF, TGF- α , Wnt3 and the Notch ligand Dll4, all essential signals for stem-cell maintenance in culture. Co-culturing of sorted stem cells with Paneth cells dramatically improves organoid formation. This Paneth cell requirement can be substituted by a pulse of exogenous

Wnt. Genetic removal of Paneth cells in vivo results in the concomitant loss of Lgr5 stem cells. In colon crypts, CD24+ cells residing between Lgr5 stem cells may represent the Paneth cell equivalents. We conclude that Lgr5 stem cells compete for essential niche signals provided by a specialized daughter cell, the Paneth cell.

Dissecting the Stem Cell Component of Precancerous Lesions

Frank McKeon

The Jackson Laboratory for Genomic Medicine, USA

Frank MacKeon and Wa Xian

The Jackson Laboratory for Genomic Medicine, Farmington, CT USA; Genome Institute of Singapore, A-STAR, Singapore; Department of Medicine, National University Health System, Singapore; Department of Genetics and Developmental Biology, University of Connecticut Health Center, USA

It is now established that epithelial cancers arise from precancerous lesions that typically emerge 20 years prior to the onset of carcinoma. Given the intractability of many of these carcinomas, much hope lies in strategies of preemptively targeting these precursor lesions. Barrett's esophagus is a precancerous lesion that confers a significant risk of esophageal adenocarcinoma. Strategies for selective eradication of Barrett's have been stymied by our inability to identify and characterize Barrett's stem cells. We are developing novel technologies to clone patient-matched stem cells of Barrett's, gastric, and esophageal epithelium. Genomic analyses of Barrett's stem cells reveal a patient-specific mutational spectrum ranging from low mutational burden similar to patient-matched gastric epithelial stem cells to ones marked by extensive alteration of genes implicated in tumor suppression, epithelial planarity, and epigenetic regulation. Transplantation of transformed Barrett's stem cells yielded tumors with hallmarks of esophageal adenocarcinoma, whereas transformed esophageal stem cells produced squamous cell carcinomas. Thus Barrett's develops from cells distinct from either the esophagus or the stomach, can emerge without obvious driver mutations, and likely progresses through and from the generation of dominant clones. These findings define a stem cell target for preemptive therapies of a precancerous lesion, and suggest similar approaches can assist therapeutic approaches to preclude other aggressive cancers.

Skin Stem Cells in Silence, Action and Cancer

Elaine Fuchs

Howard Hughes Medical Institute, The Rockefeller University, USA

Stem cells have the ability to self-renew long term and differentiate into one or more tissues. Typically, stem cells are used sparingly to replenish cells during normal homeostasis. This is particularly true of adult tissues that undergo relatively infrequent or periodic turnover. However, even stem cells that are quiescent must be able to respond quickly to injury in order to fuel rapid tissue regeneration. How stem cells balance self-renewal and differentiation is of fundamental importance to our understanding of normal tissue maintenance and wound repair.

Moreover, increasing evidence suggests that the regulatory circuitry governing this balancing act is at the root of some types of tumors both in mice and in humans.

We've been using skin as a paradigm to understand how stem cells function in normal tissue generation and how this process goes awry in cancer. We've elucidated many of the pathways involved in how extrinsic signaling mobilizes stem cells to make tissue. In so doing, we have revealed mechanisms which are also deregulated in a variety of different human cancers. This has led us to the realm of identifying cancer stem cells (tumor-initiating cells) of squamous cell carcinomas of the skin. The second most abundant form of cancer world-wide, skin squamous cell carcinomas also establish a paradigm for many life-threatening cancers of lung, esophagus, breast, cervix, prostate, throat and oral tissues. We've demonstrated that when purified and transplanted serially into the skins of host recipients, a single cancer stem cell from a skin squamous cell carcinoma can generate a new squamous cell carcinoma that is similar in properties to the parent tumor. We've also discovered that cancer stem cell numbers and aggressiveness are predicated on the tumor microenvironment and on the ability of cancer stem cells to respond to it. The striking differences between the microenvironments of cancer stem cells and their normal counterparts seems likely to contribute to why their expression profiles are also markedly distinct.

These findings underscore the importance of dissecting the molecular mechanisms of tumor progression and growth in vivo. To this end, we've developed a non-invasive method to knockdown genes rapidly, efficiently and selectively in the skin epithelium. We've exploited this technology to carry out a whole genome-wide RNAi screen in mice for genes that are selectively involved in regulating oncogenic growth in the skin. Our findings have major implications for our understanding of tumor progression and cancer.

[This work was supported by the HHMI and a grant from the National Institutes of Health]

Mouse Spermatogenic Stem Cell Self-renewal

Ben Simons

University of Cambridge, UK

In mammals, sperm production relies upon spermatogenic stem cells. In the absence of a discrete anatomical niche, stem cell identity and function has proved difficult to resolve. By combining in vivo live-imaging with pulse-labeling studies, we resolve the active migration and fate behavior of GFR α 1-expressing spermatogonia and their progeny at single-cell resolution in mouse. Using these observations to define a minimal modeling scheme, we show that the heterogeneous population of singly isolated (As) and syncytial spermatogonia together comprise a single stem cell pool. Through a coordinated process of incomplete cell division (syncytial extension) and intercellular bridge breakdown (syncytial fragmentation), stem cells continuously and reversibly transit between these different morphological categories, while

giving rise to differentiating progeny. By showing that stem cell function is shared among a dynamically interconverting heterogeneous pool, this study offers a new perspective on the maintenance of adult tissues.

Stem Cells: Lost in Translation

Sean Morrison

Children's Medical Research Institute at UT Southwestern, USA

Defects in ribosome function are known to cause haematopoietic failure. However, it is unknown whether this reflects an inability to synthesize enough protein to maintain cellular homeostasis or whether haematopoietic stem cells (HSCs) require highly regulated protein synthesis. Indeed, there are almost no data on protein synthesis in any somatic stem cell. We found that the rate of protein synthesis *in vivo* in HSCs was lower than in most other haematopoietic cells, even when comparing non-dividing cells. Dividing HSCs had lower rates of protein synthesis than other dividing haematopoietic cells. Reduced ribosomal function in Rpl24Bst/+ mice further reduced the rate of protein synthesis in HSCs and impaired HSC function. Pten deletion increased the rate of protein synthesis in HSCs but also reduced HSC function. Rpl24Bst/+ cell-autonomously rescued the effects of Pten deletion, restoring normal protein synthesis in HSCs, normal HSC function, and delaying leukemogenesis. HSCs thus depend upon a highly regulated rate of protein synthesis such that increases or decreases impair HSC function.

From Cells to Networks – Principles of Vascular Pattern Formation

Holger Gerhardt

Vascular Biology Laboratory, London Research Institute – Cancer Research UK; Vascular Patterning Laboratory, Vesalius Research Center, VIB, KU Leuven, Belgium

The formation of a hierarchically branched network of large and small blood vessels is critical for the growth and maintenance of healthy tissues. How the endothelial cells that line blood vessels orchestrate their behaviour, morphology and function to achieve functional blood vessel patterning is one of the most exciting questions in vascular biology. Using mosaic models of sprouting and vessel remodelling *in vitro*, *in silico* and *in vivo*, we investigate how endothelial cells stimulated by VEGF-A dynamically specify their behaviour and neighbourhood relationships to branch or expand new vessels. I will present new insights into the principles of vascular pattern formation, focussing on cell specification, coordination, endothelial cell competition and dynamic rearrangements during sprouting and remodelling.

SESSION III 2:00 – 5:00 PM

Cell Fate Control

Contribution of the Reprogrammed Vascular Niche to Stem Cell Self-renewal and Organ Regeneration

Shahin Rafii

Weill Cornell Medical College, Angiocrine Bioscience, Ansary Stem Cell Institute, HHMI, USA

Organ specific endothelial cells (ECs) are not just passive conduits to deliver oxygen and nutrients, but also establish an instructive vascular niche, which by elaboration of specific paracrine trophogens, (known as angiocrine factors), directly balance the rate of stem cell self-renewal and differentiation. For example, activation of Akt-mTOR pathway in the sinusoidal ECs (SECs) stimulates expression of angiocrine factors, including Notch-ligands, Wnts, FGFs and TGF-modulators, that induce expansion of authentic hematopoietic stem cells. While MAPkinase induces expression of angiocrine factors, that support differentiation of the stem cells into lineage-committed progenitors.

Furthermore, after partial hepatectomy, SECs within the liver stimulated regeneration by angiocrine expression of Wnt2 and HGF. Pulmonary capillary ECs (PCECs) by deploying MMP14 and release of EGF-ligands sustain lung regeneration. Notably, transplantation of SECs or PCECs into mice restores organ regeneration. These data establish the remarkable tissue-specific vascular heterogeneity in orchestrating organ regeneration. Indeed, we have recently shown that each organ is arborized with specialized capillary ECs endowed with unique repertoire of angiocrine factors. Therefore, to capitalize on the potential of vascular cells for organ regeneration, we need to engineer tissue-specific ECs that can home and engraft in tissues promoting organ regeneration and repair. Most importantly, as ECs can be provoked to instigate pro-fibrotic changes, we need to manufacture tissue-specific ECs that drive organ regeneration without promoting maladaptive fibrosis.

To translate these findings to the clinical setting, we have differentiated human and mouse embryonic stem and iPSC cells into induced vascular endothelial cells (iVECs). However, iVECs are unstable and have limited expansion potential. Most importantly, iVECs are plastic and tend to drift into other non-vascular, such as smooth muscle cells. To circumvent this hurdle, we have developed new strategies by transcriptional (short term ETV2+Flil+Ergl+TGFβ inhibition) reprogramming of amniotic cells into vascular ECs (rAC-VECs) without employing pluripotent transcription factors. rAC-VECs phenocopy the specialized tissue-specific function of ECs, supporting long-term expansion of repopulating cells, such as hematopoietic stem cells in xenobiotic-free conditions.

We show that once transplanted intravenously, rAC-VECs can home to the regenerating tissues and by production of specific angiocrine factors promote organ regeneration and repair without provoking aberrant fibrosis. Given that rAC-VECs can be HLA-typed, cryopreserved, and publicly banked, these cells

could establish an inventory for generating abundant tissue-specific vascular niche cells for promoting angiocrine-dependent organ regeneration.

The Role of Epithelial Progenitors in Liver Injury

Markus Grompe

Oregon Health and Science University, USA

Abstract not available at time of printing.

Reprogramming Approaches to Cardiovascular Disease

Deepak Srivastava

Gladstone Institute of Cardiovascular Disease and University of California San Francisco, USA

Heart disease is a leading cause of death in adults and children. We, and others, have described complex signaling, transcriptional and translational networks that guide early differentiation of cardiac progenitors and later morphogenetic events during cardiogenesis. We found that networks of transcription factors and miRNAs function through intersecting positive and negative feedback loops to reinforce differentiation and proliferation decisions. Many of the developmental cues have been leveraged to control differentiation of pluripotent stem cells into cardiac, endothelial and smooth muscle cells that may be useful for regenerative purposes. We have used similar approaches to reprogram disease-specific human cells in order to model human heart disease in patients carrying mutations in cardiac developmental genes. We also utilized a combination of major cardiac regulatory factors, *Gata4/Mef2c/Tbx5*, to induce direct reprogramming of cardiac fibroblasts into cardiomyocyte-like cells with global gene expression and electrical activity similar to cardiomyocytes. The *in vivo* efficiency of reprogramming into cells that are more fully reprogrammed was greater than *in vitro* and resulted in improved cardiac function after injury. We are exploring the molecular mechanisms underlying the progressive reprogramming process through the study of DNA-binding of reprogramming factors and the associated epigenetic and transcriptional changes. We have also identified a unique cocktail of factors that reprogram human fibroblasts into cardiomyocyte-like cells and are testing these in large animals. Knowledge regarding the early steps of cardiac differentiation *in vivo* has led to effective strategies to generate necessary cardiac cell types for disease-modeling and regenerative approaches, and may lead to new strategies for human heart disease

Human ES Cell Derived Midbrain Dopamine Neurons for Cell Therapy in Parkinson's Disease

Lorenz Studer

SKI Center for Stem Cell Biology, Sloan-Kettering Institute for Cancer Research, USA

Parkinson's disease (PD) is characterized by the loss of midbrain dopamine neurons that is responsible for the characteristic motor symptoms of the disease. Dopamine neuron replacement has been proposed as an experimental therapeutic strategy since the 1980s with more than 300 patients grafted world-wide using human fetal tissue. While those studies have demonstrated the feasibility of dopamine neuron replacement the clinical results have been mixed.

One main limitation of fetal tissue grafting has been the limited supply and the variable nature of tissue for transplantation. Over the last 5 years there has been renewed interest in the dopamine neuron replacement paradigm, triggered by a re-analysis of grafted patients > 10 years after transplantation suggesting remarkable long-term benefit in a subset of individuals. Furthermore, novel sources of dopamine neurons have become available to resolve issues such as limited supply or variability of the tissue for transplantation. Here we will review our data on the use of human ESC-derived dopamine neurons in mouse, rat and monkey models of PD. Directed differentiation protocols yield large numbers of authentic dopamine neurons via a human ESC floor plate intermediate. Furthermore, those dopamine neurons show excellent *in vivo* survival and function in the various PD models. Finally, we will discuss remaining challenges for translating pre-clinical findings towards clinical use and present strategies to further improve the function and safety of human ESC derived DA neurons; efforts geared towards the first clinical trial of human ESC-derived dopamine neurons in PD patients.

Towards Regenerative Medicines for Multiple Sclerosis

Robin Franklin

Wellcome Trust-MRC Cambridge Stem Cell Institute, University of Cambridge, UK

Remyelination, the process by which new myelin sheaths are restored to demyelinated axons, represents one of the most compelling examples of adult multipotent stem cells contributing to regeneration of the injured CNS. This process can occur with remarkable efficiency in multiple sclerosis (MS), and in experimental models, revealing an impressive ability of the adult CNS to repair itself. However, the inconsistency of remyelination in MS, and the loss of axonal integrity that results from its failure, makes enhancement of remyelination an important therapeutic objective. There is now compelling evidence that ageing is the major contributor to the declining efficiency of remyelination and that this is largely due to a failure of stem cell differentiation. This talk will review recent studies we have undertaken aimed at obtaining a detailed understanding of the mechanisms of regulating differentiation during remyelination and hence identifying novel therapeutic targets.

Genome Exchange in Human Oocytes

Dieter Egli

New York Stem Cell Foundation, USA

Oocytes have the ability to give rise to all cells and tissue types. The factors establishing these developmental programs segregate from the genome in meiosis, and localize to the cytoplasm of the unfertilized egg. This physical separation allows the removal of the oocyte genome without removing the factors required for development. Upon introduction of a genome from a somatic cell, the oocyte cytoplasm confers the developmental potential of the oocyte to the newly introduced genome. These basic principles of oocyte biology were discovered in animal oocytes. Similar research on human oocytes has only recently become possible as adequate guidelines enabling the donation of human oocytes for research were established. The exchange of the genome in human oocytes not only informs us about the requirements for normal embryonic development, but may also be of therapeutic relevance. Exchanging the oocyte genome with the somatic cell of a patient to generate embryonic stem cells may be useful to develop replacement cells, such as beta cells for diabetes. Furthermore, exchanging the oocyte genome with the genome of another oocyte may be useful to prevent the transmission of mitochondrial disorders. Mitochondrial diseases can be caused by mutant mitochondrial DNA transmitted within the cytoplasm of the egg from mother to child. The transfer will result in the elimination of mutant mitochondrial DNA. Here I will present an update on our research on genome exchange in human oocytes.

Sponsor supported presentation

Flow Cytometry Applications for Isolating and Analyzing Complex Heterogeneous Stem Cell Cultures

Mirko Corselli

R&D Scientist, BD Biosciences, USA

The differentiation of stem cells often yields inconsistent and heterogeneous cell populations that are problematic for transplantation and quantitative and comparative analyses. One way to address this challenge is to identify unique combinations of markers to facilitate the isolation of near-pure cell populations from heterogeneous cell cultures. In addition, there is a need for robust and standardized flow cytometry tools and methods for analyzing heterogeneous stem cell cultures. Applications discussed in this seminar will include:

- Cell surface marker screening for the isolation of hESC-derived neurons by FACS
- Tips and tricks for efficient cell sorting of hESC, hiPSC, NSC and neurons
- Quantification of differentiation status by flow cytometry
- Assessment of MSC phenotype by flow cytometry

Tuesday, September 17

SESSION IV 9:00 AM – 12:00 PM

Cell Therapy

Self-organization of Neural Structures in 3D Stem Cell Culture

Yoshiki Sasai

Center for Developmental Biology RIKEN, Japan

Over the last several years, much progress has been made for in vitro culture of mouse and human ES cells. Our laboratory focuses on the molecular and cellular mechanisms of neural differentiation from pluripotent cells. Pluripotent cells first become committed to the ectodermal fate and subsequently differentiate into uncommitted neuroectodermal cells. Both previous mammalian and amphibian studies on pluripotent cells have indicated that the neural fate is a sort of the basal direction of the differentiation of these cells while mesoendodermal differentiation requires extrinsic inductive signals. ES cells differentiate into neuroectodermal cells with a rostral-most character (telencephalon and hypothalamus) when they are cultured in the absence of strong patterning signals. In this talk, I first discuss this issue by referring to our recent data on the mechanism of spontaneous neural differentiation in serum-free culture of mouse ES cells. Then, I will talk about self-organization phenomena observed in 3D culture of ES cells, which lead to tissue-autonomous formation of regional structures such as layered cortical tissues. I also discuss our new attempt to monitor these in vitro morphogenetic processes by live imaging, in particular, self-organizing morphogenesis of the optic cup in three-dimensional cultures. Self-driven mechanisms by internal forces and local changes in mechanical property govern the complex invagination process during early eye development.

Regenerative Medicine From Bench to Beside: Overcoming the Challenges to Restore Vision

Graziella Pellegrini

University of Modena and Reggio Emilia, Italy

Limbal cultures restore the corneal epithelium in patients with ocular burns. We investigate biological parameters instrumental for their clinical success. We report a long-term multicenter prospective study on 152 patients, carrying severe burn-dependent corneal destruction, treated with autologous limbal cells cultured on fibrin and clinical-grade 3T3-J2 feeder cells. Clinical results were statistically evaluated both by parametric and non-parametric methods. Clinical outcomes were scored as full success, partial success and failure in 66.05%, 19.14%, and 14.81% of eyes, respectively. Total number of clonogenic cells, colony size, growth rate and presence of conjunctival cells could not predict clinical results. Instead, clinical data provided conclusive evidence that graft quality and likelihood of a successful outcome rely on an accurate evaluation of

the number of stem cells detected before transplantation as holoclones expressing high levels of the p63 transcription factor. No adverse effects related to the feeder-layer has been observed and the regenerated epithelium was completely devoid of any 3T3 contamination. Cultures of limbal stem cells can be safely used to successfully treat massive destruction of the human cornea. We emphasize the importance of a discipline for defining the suitability and the quality of cultured epithelial grafts, which are relevant to the future clinical use of any cultured cell type.

Interaction Between Mesenchymal Stromal Cells, Bioengineered Tissues and Organs, and Human Body

Paolo Macchiarini

Advanced Center of Translational Regenerative Medicine Karolinska Institute, Sweden

Abstract not available at time of printing.

A Phase I/II Cell Therapy Trial for Duchenne Muscular Dystrophy

Gulio Cusso

University College London, UK

Napolitano S¹, Cicalese MP¹, Previtali S¹, Marktel S¹, Venturini M¹, Politi L¹, Noviello M. ¹, Tedesco FS^{1,2}, Bonini, C¹., Torrente Y³, Ciceri F¹ and Cossu G^{1,2}

¹Division of Regenerative Medicine, Department of Neurology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, ²Department of Cell and Developmental Biology, University College London, ³Department of Neurological Sciences, University of Milan.

Mesoangioblasts are progenitor cells, associated with the vasculature and able to differentiate into different types of mesoderm, including skeletal muscle (Minasi et al. Development 129, 2773, 2002). When mesoangioblasts were delivered intra-arterially to muscles of dystrophic mice and dogs they resulted in a significant functional amelioration (Sampaoli et al. Science 301, 487, 2003; Nature 444, 574, 2006). Human adult mesoangioblasts, isolated and expanded in vitro from muscle biopsies, were shown to correspond to a subset of pericytes (Dellavalle et al. Nature Cell Biol. 9, 255, 2007).

Based on these results, a mono-centre, prospective, non-randomized, clinical phase I/II study of cell therapy with HLA-matched donor human mesoangioblasts in DMD patients started in June 2009, with a one year preliminary study (involving 28 DMD patients, aged 5-10), required to validate outcome measures. Starting on March 2011, three out of these patients (with an HLA-identical donor) underwent successive intra-arterial transplantations at escalating doses of cells, under a continuous regime of immune suppression. Two more patients are being currently treated. Safety was the primary objective of the study. A possible modification in muscle strength as a consequence of mesoangioblast transplantation is also being evaluated. The results of this trial will be presented.

Moreover, limitations of the current strategy and possible solutions to overcome them will also be discussed.

This work was supported by grants from the European Community (Optistem), the European Research Council, Duchenne Parent Project, Telethon, CureDuchenne, AFM and the Italian Ministries of Research and Health.

Cell Based Therapeutics for Skeletal Applications

Frank Luyten

Skeletal Biology and Engineering Research Center & Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, Belgium

The clinical impact of cell based therapeutic approaches for applications in the field of skeletal tissue regeneration is still limited. More research and development is needed on many aspects of these "Advanced Therapeutic Medicinal Products" including mechanisms of action, choice of cell type and scaffolds and manufacturing technologies.

In an effort to optimize tissue engineering technologies, we have been exploring many of the challenges in a coordinated fashion with a focus on bone engineering, from bench to bedside.

Our studies include the rational selection of components for a combination product including cells, growth factors and scaffold materials based on their in vivo bone formation and bone remodelling. We found that the cell-factor-material combinations behaved quite differently in vivo, despite apparent in vitro similarities in cell proliferation and differentiation. Our data highlight the importance of matching a biological matrix with stem cell type and growth factor, and identify parameters that can be used for the rational selection of biomaterials in combination products for bone engineering.

We also introduced the "developmental engineering" methodology for the design of robust manufacturing processes. This methodology is based on the design of ex vivo processes consisting of sequential sub-processes corresponding to in vivo developmental stages. They follow a gradual and coordinated progression of tissue growth and cell differentiation that leads to organization of cells into intermediate tissue forms. The macroscopic developmental modularity of tissues can be attributed to a corresponding modularity of the network topology that describes gene interactions during the developmental process. Microarray studies with a variety of analysis approaches such as gene expression dynamics and principal component analysis, gene clustering and osteogenic network analysis provide input on the factors/pathways critical in driving the bone formation processes.

In conclusion, a second wave of new and more rational approaches for tissue engineering in general, and bone repair in particular, will hopefully lead to more predictable clinical outcomes.

Secreted Factors and MicroRNAs Inducing Cardiac Regeneration

Mauro Giacca

International Centre for Genetic Engineering and Biotechnology, Italy

There is an impelling need to develop novel therapeutic strategies aimed at inducing cardiac repair and regeneration in patients with myocardial infarction and heart failure. In contrast to other species that regenerate the heart during the adult life, damage to the myocardium in mammals is repaired through the formation of a scar. However, multiple evidence now indicates that a limited capacity of myocardial renewal might also exist in adult individuals; thus, we are actively searching for factors able to foster this regenerative capacity. A first approach entails direct myocardial gene transfer using viral vectors based on the Adeno-Associated Virus (AAV), which transduce the heart at very high efficiency. Using these vectors, we are undertaking an exhaustive approach to identify factors promoting cardiac repair, selected from an AAV library corresponding to the mouse secretome (1600+ secreted proteins). A second approach entails high throughput screening of microRNAs promoting cardiomyocyte proliferation. Starting from a whole genome human microRNA library, we identified a few microRNAs endowed with an outstanding activity of promoting expansion of cardiomyocytes in cell culture, inducing massive cardiac hyperplasty in the neonatal heart and improving cardiac function after myocardial infarction in the adult. These microRNA acts by directly activating the proliferative potential of differentiated cardiomyocytes, thus bypassing the requirement of stem cell expansion and differentiation.

SESSION V 2:00 – 5:00 PM

Gene Therapy

Gene Therapy for Severe Immunodeficiencies

Bobby Gaspar

Centre for Immunodeficiency, UCL Institute of Child Health, UK

Primary immunodeficiencies have played a major role in the development of gene therapy for monogenic diseases of the bone marrow. The last decade has seen convincing evidence of long term disease correction as a result of ex vivo viral vector mediated gene transfer into autologous haematopoietic stem cells. The success of these early studies has been balanced by the development of vector related insertional mutagenic events. More recently the use of alternative vector designs with self inactivating (SIN) designs which have an improved safety profile has led to the initiation of a wave of new studies which are showing early signs of efficacy. These studies in SCID-X1, ADA-SCID, Wiskott-Aldrich syndrome and Chronic Granulomatous disease are all multi-centre studies using lentiviral vectors and have the potential to recruit patients rapidly and to show efficacy and safety. The on-going development of safer vector platforms and gene editing technologies together with improvements in cell transduction techniques and optimised conditioning regimes is likely to make gene therapy

amenable for a greater number of PIDs. If long term efficacy and safety are shown, gene therapy will become a standard treatment option for specific forms of PID. These technologies may also be important for other monogenic disorders of the haematopoietic system.

Uncovering Hematopoietic Stem Cell Dynamics and Activity in vivo through Vector Insertion Site Barcoding in Humans

Luca Biasco

HSR-TIGET, San Raffaele Telethon Institute for Gene Therapy/Division of Regenerative Medicine, Stem Cells and Gene Therapy, San Raffaele Scientific Institute, Italy

Luca Biasco¹, Serena Scala¹, Cristina Baricordi¹, Francesca Dionisio¹, Andrea Calabria¹, Samantha Scaramuzza¹, Nicoletta Cieri², Danilo Pellin³, Clelia Di Serio^{3,5}, Cynthia Bartholomae⁴, Chiara Bonini², Christof Von Kalle⁴, Manfred Schmidt⁴, Luigi Naldini^{1,5}, Eugenio Montini¹, and Alessandro Aiuti^{1,6}

¹ San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Milano, Italy; ² San Raffaele Scientific Institute, HSR, Italy; ³ CUSSEB, Università Vita-Salute, Milan Italy; ⁴ National Center for Tumor Diseases (NCT-DKFZ), Heidelberg, Germany; ⁵ Univ. Vita-Salute San Raffaele, Milano, Italy; ⁶ Univ. of Rome "Tor Vergata", Rome, Italy.

The hematopoietic system is a complex hierarchical structure composed by a variety of blood cell types with a wide spectrum of different functions essential to human physiology. However, the dynamics and fate of human hematopoietic stem progenitor cells (HSC) and their progeny are currently studied only through surrogate in vitro assays or animal models. Upon retroviral-mediated gene correction, transduced cells become univocally marked by vector integration sites (IS). Thus, our gene therapy (GT) clinical trials for adenosine deaminase (ADA) deficient-SCID and Wiskott-Aldrich Syndrome (WAS), based on the infusion of retrovirally engineered HSC, constitute a unique setting to study human hematopoietic reconstitution after transplant as well as long-term activity of HSC in vivo through IS molecular tracking. To this aim, we collected 32.363 IS from purified cell lineages in 3 WAS patients treated with lentiviral vector GT up to 1.5 years and 4.845 IS in 4 ADA-SCID patients treated with gammaretroviral GT up to 6 years after treatment.

In WAS GT patients, we uncovered the presence of self-limiting waves of individual progenitor activity during the first phases after transplant, stabilizing at 12-18 months where the diversity of clonal repertoire reached a plateau. Multipotent HSC active in WAS patients could be identified overtime by the detection of identical IS in HSC, colony forming units and peripheral blood mature cells. Of note, the highest proportion of shared identical IS (38.5%) was found in clonogenic progenitors, validating the biological consistency of IS analysis and the colony assays as representative of in vivo HSC activity. By mathematical estimators, we also calculated a theoretical minimal number of about 1,700-6,300 transduced active stem cells in our patients corresponding to 1 in 3×10^5 infused CD34+ cells.

In ADA-SCID patients we showed that identical IS are consistently detected at multiple lineages level even several years after GT. Since gammaretroviral transduction occurs only in actively

replicating cells, this finding provides the first evidence that in vitro stimulated HSC, “awaken” from dormancy, can still retain in vivo long-term activity in humans. By semi-quantitative PCRs on specific vector-genome junctions we tracked a fluctuating but consistent output of marked HSC over a period of 5 years. We were also able to apply network-based statistical approaches to IS similarity among lineages testing the validity of the different proposed models of hematopoietic hierarchy.

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. We phenotypically and functionally identified these cells as the recently described T memory stem cells (TSCM) carrying naïve plasticity and memory survival. We are currently retrieving crucial information on safety and efficacy of TSCM engineering in humans, useful for their potential exploitation in the development of T-cell based anti-tumor strategies.

In summary our unique molecular tracking of individual hematopoietic clones provides new evidences on stem cell activity and dynamics in vivo in humans, relevant for the design of therapeutic approaches for a broad spectrum of hematological diseases and tumors.

Adaptive Immunodeficiency and Gene Therapy

Alain Fischer
CHU Necker INSERM U768, France

Over the last 20 years, the genetics and pathophysiology of many primary immunodeficiencies (PID) have been unraveled. This offers the opportunity to treat the most severe of these conditions by gene transfer into hematopoietic stem cells. Primary T cell immunodeficiencies appear as the best therapeutic targets because of the expected selective growth advantage conferred by gene expression into T cell precursors combined with the very long life span of T lymphocytes. Based on this rationale, ex vivo retroviral gene transfer into hematopoietic progenitors has been shown to lead to sustained (over 14 years) correction of severe combined immunodeficiency (SCID). The advent of genotoxicity events in some patients has initiated the development of modified vectors, i.e. “SIN vector” in use over the last 3 years with efficacy and safety to treat SCID as well as other PID such as the Wiskott Aldrich syndrome.

Preclinical Genotoxicity Testing of Stem Cell Products

Christopher Baum
Hannover Medical School, Germany

Novel stem cell products open important treatment options for patients suffering from serious diseases. Proof of concept is available for an increasing number of clinical entities. However, an important safety concern addresses the risk of mutations;

these may pre-exist in the original cell population or be acquired via culture adaptation or therapeutic gene transfer (“genotoxicity” in sensu stricto). Major issues encountered in the preclinical safety testing of stem cell products are the rarity of the mutants that may give rise to adverse events, and the long latency of disease initiated by such mutants. Many preclinical assays developed to detect potentially dangerous mutants are still limited in their sensitivity and do not yet follow the standards of quality control established for industry-driven pharmaceutical developments. To share resources, drive innovation and improve quality control at all levels, large international academic consortia have been formed. Focusing on the genetic modification of hematopoietic cells and induced pluripotent stem cells for the treatment of congenital disorders of blood formation, I will describe recent developments of assays for preclinical safety determination of stem cell products, highlighting the importance and remaining difficulties encountered with genetic tracking studies to assess clonal cell identity and the development of stem cell progeny in vivo.

Endogenous Retroelements, KRAB’n’KAP, and the Preservation of Stem Cell Transcriptional Dynamics

Didier Trono
École Polytechnique Fédérale de Lausanne EPFL, Switzerland

Endogenous retroelements (EREs) account for more than half of the human genome, and although they constitute essential motors of evolution, they can occasionally cause disease. Accordingly, they are inactivated through epigenetic mechanisms during the earliest stages of embryogenesis. I will describe how this process is mediated by a large family of transcriptional repressors, the tetrapod-specific KRAB-containing zinc finger proteins (KRAB-ZFPs), and by their cofactor KAP1 (KRAB-associated protein 1). I will further illustrate how KRAB/KAP1-mediated regulation is responsible for silencing a very broad range of EREs in human embryonic stem (ES) cells, in an evolutionally dynamic fashion, and how it exerts as a consequence a marked influence on the transcriptional landscape of these cells. Finally, I will present our analysis of the transcriptional activity of EREs during the reprogramming of somatic cells to induced pluripotent stem cells (iPSCs), and discuss how its noted alterations might contribute to the inefficiency of the reprogramming process, and how the incomplete control of individual ERE integrants may explain the noted phenotypic heterogeneity of iPSCs.

Genetic Engineering of Hematopoietic Stem Cells

Luigi Naldini
San Raffaele Scientific Institute, Italy

Abstract not available at time of printing.

Wednesday, September 18

SESSION VI 9:00 AM – 12:00 PM**Defining Regenerative Mechanism****Commercial Stem Cells: The lesson of the “MSC” case**

Paolo Bianco

Sapienza University of Rome, Italy

Stem cell research is not only at the forefront of biomedical science in a technical and conceptual way; it is also one of the areas where a novel layout of the interaction between science and industry finds a direct and congruent application. The field of so-called “mesenchymal stem cells” however exemplifies a potential “dark side” of such novel type of interaction. In this field, a significant proportion of the literary output over the past 15 years at least has been directly influenced by commercially driven science. This has produced a remarkable divergence of two distinct descriptions of the same objects of the natural world (stem cells and diseases for which they could be of interest). One is science-based, and consists of sets of data reproduced extensively in multiple laboratories around the world, and now transitioning in established knowledge. The other is commerce-based, does not rest on widely reproduced data, assays, or results, and yet has pervaded a large proportion of the literature. In one “view”, the object called “MSCs” is a self-renewing, multipotent progenitor of skeletal tissues found in the wall of bone marrow sinusoids, assayable at the single cell level, locally transplantable and capable of establishing bone and the hematopoietic microenvironment. In the other view, not supported by positive, widely reproduced data, MSCs are ubiquitous progenitors, acting as a source of paracrine factors, systemically injectable, providing non-specific “cure” of hundreds of unrelated diseases, mostly lethal and orphan, of diverse nature. The influence of marketing strategies on the content and format of a significant literary production is easy to reconstruct at this time; a kind of “advertisement” permeating the scientific literature is obvious. Approaches to therapy that are not science-based but may be commercially of interest are proposed and extensively tested in costly and empirical clinical trials. This has several important drawbacks: 1) it indirectly opens the door to the unauthorized dissemination of unproven stem cell therapies, of which blatant cases have started to emerge in Europe and North America, often providing a “rogue” version of the “commercial” notion of MSCs and their use; 2) it generates a dangerous push for the weakening or abrogation of regulations and regulatory bodies; 3) it creates a “parallel” description of the natural world, moving, paradoxically, translation of science away from science; 4) it generates a kind of medicine at the border between fringe medicine and outright charlatanism; 5) it generates risks for patients while de facto obstructing science and scientific progress. While the feeling that much attention to “translational medicine” can accelerate medical progress, awareness is starting to emerge that an obstruction can rather result in some circumstances. This has general implications for the general model of interaction between science and the economy that is prevalent

worldwide, of specific significance in the stem cell field.

Molecular Regulation of Skeletal Muscle Stem Cells

Shahragim Tajbakhsh

Institut Pasteur, Stem Cells & Development, France

The regulatory cell state of muscle stem cells fluctuates during perinatal growth, after entering quiescence, and following muscle injury when amplification of the stem cell pool results in the production of myoblasts and future self-renewing cells. We have observed heterogeneity properties in the muscle stem cell pool during quiescence and proliferation and performed serial transplantations of subpopulations of these cells isolated directly from the quiescent state. A major regulator of cellular quiescence in this tissue is Notch signaling, which is lost rapidly following cell cycle reentry, then restored during self-renewal. We and others have reported that Notch signaling could mediate the stability of the niche by regulating extracellular matrix molecules. These observations will be discussed in the context of maintenance of the stem cell state postnatally and robustness in transplantation assays.

Dissecting the Stem Cell Niche for Therapeutic Targeting

David Sassoon

INSERM, Université de Pierre et Marie Curie-Sorbonne Universités, France

Luigi Formicola, Alice Pannérec, Vanessa Besson, Giovanna Marazzi and David A. Sassoon, UMR S 787 – Université Pierre et Marie Curie / INSERM, Faculté de Médecine Pitié-Salpêtrière, France

PW1/Peg3 is a parentally imprinted gene expressed in stem cells and progenitor populations in many murine postnatal tissues, including skeletal muscle, skin, gut, bone marrow and nervous system. We have shown that PW1/Peg3 expression correlates with adult stem/progenitor cell competence and plays a role in stem cell quiescence.

In postnatal skeletal muscle, PW1/Peg3 is expressed in satellite cells and a subset of interstitial cells (PICs, PW1+ Interstitial Cells). We demonstrated recently that juvenile and adult PICs are more plastic than satellite cells, as they are able to differentiate into smooth and skeletal muscle as well as fat, while satellite cells exhibit a committed skeletal muscle fate. Moreover, PICs can be separated into 2 sub-populations on the basis of platelet derived growth factor receptor alpha (PDGFR α) expression: PW1+PDGFR α + with adipogenic capacity and PW1+PDGFR α - with myogenic capacity.

Molecules from the TGF β family as well as insulin-like growth factor-1 (IGF-1) are involved in regulating postnatal muscle growth and homeostasis. Micro-array profiling revealed that PICs and satellite cells reciprocally express ligands and their cognate receptors in the TGF β and IGF pathways. PICs can block the inhibitory effects of myostatin on satellite cells proliferation in vitro through release of antagonist factors, including follistatin and IGF-1, demonstrating cellular crosstalk within the

muscle stem cell niche. Administering an inhibitor of the TGF β pathway *in vivo* acts on the stem cell niche by altering the ratio between PICs and satellite cells and restores the regenerative capacity in satellite-cell depleted muscles. We propose that targeting the stem cell niche allows a 'sub-threshold' number of muscle progenitors to properly execute the regenerative process and overcome environmental cues leading to fibrosis and ectopic fat deposition.

These and other data will be presented.

Developing Human RPE Stem Cells for Retinal Repair

Sally Temple

Neural Stem Cell Institute, USA

The retinal pigment epithelium (RPE) is a polarized monolayer of pigmented cells at the back of the eye, lying next to the layer of photoreceptors. The RPE is essential for photoreceptor renewal and neural retinal function and survival. Degeneration of the RPE results in blinding diseases such as age-related macular degeneration and retinitis pigmentosa. Exploring ways to regenerate the RPE could provide new opportunities for vision preservation and restoration, to combat diseases that currently have no effective therapeutic options.

During embryogenesis, the RPE is one of the earliest CNS tissues to differentiate, and it remains normally dormant throughout life. We showed recently that the adult human RPE contains a sub-population of cells that can be activated *in vitro* to proliferate extensively and differentiate to reform a cobblestone RPE monolayer. Such cells are present throughout life, even in nonagenarians. This discovery provides a new opportunity to develop therapies for retinal repair.

Disease Modeling with iPS Cells

George Q. Daley

Boston Children's Hospital, Harvard Stem Cell Institute, Howard Hughes Medical Institute, USA

A major goal of stem cell research is the creation of personalized, patient-specific stem cells for use in disease modeling and as a foundation for gene repair in the context of autologous cell therapy. The ability to restore pluripotency to somatic cells through the ectopic co-expression of reprogramming factors has created powerful new opportunities for modeling human diseases. Some skepticism remains, however; whether subtle differences might impact research applications and therapeutic potential. We have derived induced pluripotent stem cells (iPSC) from patients with a variety of genetic bone marrow failure disorders, including Dyskeratosis Congenita, Fanconi's anemia, Shwachman-Diamond Syndrome, and Diamond Blackfan Anemia. Hematopoietic differentiation of these lines *in vitro* recapitulates certain aspects of these diseases and enables novel insights into disease mechanisms, thereby confirming their utility in disease modeling for studies of pathogenesis. Aspects of these studies highlighting advantages and limitations of iPS-based disease modeling will be presented.

The Control of Hepatic Progenitor Cells for Liver Regeneration

Stuart Forbes

University of Edinburgh, UK

Following acute liver injury or partial hepatectomy the liver rapidly regenerates through the proliferation of normally quiescent hepatocytes. However, during chronic injury hepatocyte regeneration becomes impaired and a population of bipotential hepatic progenitor cells (HPCs) become activated to regenerate both cholangiocytes and hepatocytes, although their functional significance is not well defined. We have analysed their function in an inducible genetic model of liver injury: p450 dependent MDM2 deletion from differentiated hepatocytes which induces hepatocyte senescence and death. This results in massive HPC activation; these HPCs are genetically normal, bipotential, repopulate the liver and are transplantable making them a suitable therapeutic target. Understand the controls of HPC mediated regeneration are therefore important. In human diseased liver and mouse models Notch and Wnt pathways are activated in biliary and hepatocellular injury respectively. Macrophages activate HPCs in a TWEAK/fn14 dependent manner and promote their hepatocyte differentiation. In models of chronic liver injury macrophage injections result in hepatic chemokine up-regulation, recruitment of endogenous macrophages, increased MMP -13 and -9 expression in the liver; reducing fibrosis and improving liver function. We are therefore developing autologous macrophage therapy in man for the treatment of liver cirrhosis using apheresis of peripheral monocytes as a source of cells that are differentiated *ex vivo* into "regenerative macrophages".

SESSION VII 2:00 – 5:30 PM

Lineage Epigenetics

Gene Regulatory Networks Governing Hematopoietic Stem Cell Development and Identity

Tariq Enver

UCL Cancer Institute, University College London, UK

Several studies have addressed questions about transcriptional regulation within particular hematopoietic cell compartments. Few, however, have attempted to capture the transcriptional changes that occur during the dynamic transition from one compartment to another. We have profiled gene expression as multipotential progenitors underwent commitment and differentiation to two alternative lineages, focusing on the first 3 days of differentiation when the majority of decisions about cell fate are made. We have combined this with genome-wide identification of the targets of three key transcription factors before and after differentiation; GATA-2, usually associated with the stem/progenitor compartment; GATA-1 (erythroid); and PU.1 (myeloid). We used correlation analyses to associate transcription factor binding with particular modules of co-

expressed genes, alongside detailed sequence analysis of bound regions. Data-driven dynamical modelling of TF relationships has predicted novel interactions that have been validated experimentally. These approaches have highlighted novel regulators of stem cell fate decisions and - informed our understanding of GATA factor switching. Overall, the data reveal greater degree of complexity in the interplay between GATA-1, 2 and PU.1 - in regulating hematopoiesis than has hitherto been described, and highlights the importance of a genome-wide approach to understanding complex regulatory systems. A significant challenge in the field is how to relate these types of population-based data to the action of transcriptional regulators within single cells where cell fate decisions ultimately are effected. As a step toward this, we have generated single cell profiles of gene expression for a limited set of transcriptional regulators in self-renewing and committed blood cells. Based on these data, we implemented a stochastic computational model which captures mechanistic aspects of transcriptional regulation for putative commitment-associated genes and affords in silico exploration of commitment scenarios. Simulations highlight different transcriptional regulatory modes underlying heterogeneous gene expression and relate these parameters with frequency of commitment. Our model suggests that individual cells may enter lineage commitment through different routes, and experimental data on instruction of commitment through perturbation of individual regulators is in support of this view.

Targeting Developmental Pathways in Cancer Cells and Stem Cells

Frederic de Sauvage

Genentech, Inc., USA

The intestinal epithelium has a remarkable capacity for continual self-renewal; cells born in the proliferative crypt zone migrate upwards and are lost from villus tips an average of 5 days after birth. Multiple stem cell compartments, including crypt based columnar cells, the called “+4” cells or cells in other more committed compartments, appear to contribute to intestinal homeostasis and regeneration. However, the respective contribution and interplay between these cells remain poorly understood. The Notch, Wnt and Hedgehog signaling pathways play critical roles during embryonic development. These factors modulate proliferation or differentiation of numerous cell types and are also involved in the regulation of the self-renewal and/or differentiation of embryonic and adult stem cells. These pathways can also be involved in tumorigenesis when reactivated in adult tissues through mutations or overexpression of pathway components. In particular, all 3 pathways play a role in normal gut homeostasis and may be involved in some aspect of intestinal tumorigenesis. The development of inhibitors targeting these pathways is therefore of the highest interest. We will discuss the role these developmental pathways in regulating intestinal stem cell populations in normal and tumor tissues.

Epigenetic Mechanisms Regulating Epidermal Stem Cell Function

Salvador Aznar-Benitah

Institute for Biomedical Research, Spain

Adult tissues constantly self-renew to ensure homeostasis. Tissue maintenance and repair upon damage depend primarily on a population of adult stem cells that reside in specialized niches within the tissue. To replenish lost or damaged cells, adult stem cells must divide in a self-renewing manner; exit their niche, and enter the terminal differentiation program in a concerted manner. Failure to balance these events predisposes the tissue to premature aging, loss of regenerative capacity, or developing carcinomas. We have performed a genetic analysis during the stepwise differentiation of epidermal stem cells to terminally differentiated cells to identify chromatin-remodelling factors that might regulate different aspects of epidermal stem cell function. In this screen we have identified Jarid2, Cbx4 and DNA methyltransferases (Dnmt3a and Dnmt3b) as candidate genes relevant for the transition from dormancy to activation, as well differentiation. I will present data on the in vivo function of these pathways in human and mouse epidermal stem cells, obtained from conditional mouse models and genomewide analyses. I will discuss the relevance of these pathways in adult tissue homeostasis and aging.

Intratumoural Heterogeneity in Human Serous Ovarian Carcinoma

John Stingl

Cancer Research UK Cambridge Research Institute, UK

Siru Virtanen, James D. Brenton and John Stingl,
Cancer Research UK Cambridge Institute, University of Cambridge, Li Ka Shing Centre, Robinson Way, Cambridge, UK

To investigate the hierarchical organization of cells within human high grade serous ovarian carcinomas (HGSOC), we used a combination of multi-parameter flow cytometry and in vitro and in vivo functional assays to interrogate the growth and differentiation properties of phenotypically distinct cell populations present within 86 freshly isolated, non-cultured HGSOC tissues and ascites. Our results indicate that 3 functionally distinct subpopulations of epithelial cells can be identified. One population expresses high levels of the epithelial cell protein EpCAM, whereas the other two populations are EpCAM- and differ in the types of epithelial progeny they can generate. All detectable growth is restricted to the two EpCAM- cell populations demonstrated using in vitro colony-forming cell and sphere-forming assays. Xenotransplantation of non-sorted and flow-sorted cell populations into NSG mice suggests that optimal engraftment requires the presence of multiple cell types since individually isolated cell populations are non-tumourigenic. Preliminary results demonstrate that cisplatin treatment of human ovarian xenografts promotes the outgrowth of EpCAM- cells. Results from ongoing xenograft experiments in which the different subpopulations of cells are tagged with lentiviral reporters will be presented.

Regulation of Hematopoietic Stem Cells by *de novo* DNA Methyltransferases

Margaret Goodell

Baylor College of Medicine, USA

DNA methylation plays a central role in regulating gene expression during development, and is known to be disturbed in a variety of malignancies. The mechanisms through which aberrant DNA methylation contributes to malignancy development, and through which hypomethylating agents exert their effects, are poorly understood. We have studied *de novo* DNA methylation in murine hematopoietic stem cells (HSCs) as a model to address some of these fundamental questions. Murine HSCs express high levels of both *de novo* DNA methyltransferases Dnmt3a and Dnmt3b. Using conditional knockout mice, we have focused on the role of Dnmt3a in murine hematopoiesis, observing that in the absence of Dnmt3a, HSC self-renewal is dramatically enhanced at the expense of differentiation. Serial stem cell transplantation augments this effect, such that phenotypically normal HSCs that fail to differentiate accumulate to high levels. Paradoxically, DNA methylation, examined genome-wide, was both increased and decreased in Dnmt3a knockout HSCs, with CpG islands preferentially hyper-methylated, similar to the pattern of DNA methylation alterations in malignancies. The differentiated progeny of Dnmt3a knockout HSCs exhibited aberrant continued expression of stem cell-specific genes that are normally repressed during differentiation. While mutations in DNMT3A are prevalent in human acute myeloid leukemia, we observed no frank leukemia developing in the mice within the timeframe initially examined. We have now examined HSC-specific Dnmt3b knockout mice, and Dnmt3a-Dnmt3b double knockout HSCs. In absence of both *de novo* DNA methyltransferases, the HSCs accumulate even more dramatically than in the Dnmt3a knockout, even though loss of Dnmt3b alone has minimal impact. Introduction of oncogenes into the Dnmt3a knockout HSCs decreases the time to malignant transformation compared to the oncogene or Dnmt3a knockout alone. We will discuss these recent observations, along with the implications these findings have for understanding mutations of DNA methyltransferases found in human hematologic malignancies.

Directed Differentiation of Human Pluripotent Stem Cells

Gordon Keller

McEwen Centre for Regenerative Medicine and Princess Margaret Cancer Centre, University Health Network, Canada

The efficient generation of differentiated cell types from human pluripotent stem cells is dependent on recapitulating the key stages of embryonic development *in vitro*, including the formation of a primitive streak-like (PS) population, the induction of the appropriate germ layer and the specification of this germ layer to the desired lineage. These developmental steps are controlled in part, by precise stage-specific activation and inhibition of different signaling pathways including activin/nodal, BMP/Wnt, FGF and VEGF/KDR. The first stage of dif-

ferentiation, the formation of the appropriate PS population is largely dependent on the level of activin/nodal signaling, with high levels favoring the development of anterior streak cells that will give rise to definitive endoderm and intermediate to low levels promoting the generation of mid and posterior streak populations that give rise to mesoderm. When induced with high concentrations of activin, human embryonic stem cells (hESCs) will generate embryoid bodies (EBs) comprised predominantly (>90%) of anterior PS/endoderm, characterized by co-expression of CXCR4 and C-KIT. More than 70% of these cells express FOXA2 and greater than 80% are SOX17 positive. Induction of CXCR4+C-KIT+ cells with BMP-4 and bFGF results in the generation of a population of hepatic progenitors that express albumin, alpha-fetoprotein and HNF4a. When cultured further as 3D aggregates and induced with cAMP, these progenitors mature and give rise to hepatocyte-like cells that display characteristics of primary hepatocytes in the adult liver. If, on the other hand, the anterior PS cells are patterned with FGF10 and then induced with a combination of retinoic acid, EGF and nicotinamide, they differentiate along the pancreatic lineage, giving rise to PDX-1+NKX6.1+ progenitors that are able to generate monohormonal insulin-producing cells following transplantation into immunocompromised mice. Induction of hESCs with BMP-4 and low levels of activin promotes posterior streak formation and the development of hematopoietic mesoderm that is able to generate both primitive and definitive KDR+ hematopoietic progenitors. Specification of the primitive and definitive hematopoietic fates within this mesoderm population is controlled, in part, by a combination of Wnt and activin/nodal signaling. Increased concentrations of activin together with BMP-4 induce a distinct cardiovascular mesoderm population from hESCs that can be identified by the co-expression of KDR and Pdgfra. Specification of this mesoderm to a cardiovascular fate is dependent on appropriate staged inhibition of the beta-catenin/Wnt pathway. Together, these findings highlight the importance of stage specific manipulation of different signaling pathways and quantitative assessment of developing populations for the efficient and reproducible generation of differentiated cell types from pluripotent stem cells.

POSTER ABSTRACTS

POSTER PRESENTATIONS:
MONDAY, SEPTEMBER 16
5:30 – 7:00 PM

Poster Board Number: I01

NOVEL INHIBITORS OF CRM1/XPO1 NUCLEAR EXPORTER EXHIBIT STRIKING ANTI-LEUKEMIC ACTIVITY AGAINST AML AND T-ALL CELLS, INCLUDING AML LEUKEMIA INITIATING CELLS, WHILE SPARING NORMAL HEMATOPOIETIC CELLS

Etchin, Julia¹, Le, Bonnie T.¹, Kentsis, Alex¹, Sanda, Takaomi¹, Stone, Richard M.¹, McCauley, Dilara², Kauffman, Michael², Shacham, Sharon², Wang, Jean³, Kung, Andrew L.⁴, Look, A. Thomas¹

¹Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA, USA, ²Karyopharm Therapeutics, Natick, MA, USA, ³Princess Margaret Hospital/the Ontario Cancer Institute, University Health Network, Toronto, ON, Canada, ⁴Department of Pediatrics, Columbia University Medical Center, New York, NY, USA

Current treatments for acute myeloid leukemia (AML) and T-cell acute lymphoblastic leukemia (T-ALL) often fail to induce long-term remissions and are also toxic to normal tissues, prompting the need to develop new targeted therapies. One attractive cellular pathway with therapeutic potential is nuclear export, which is mediated in part by nuclear exporter CRM1/XPO1. XPO1 mediates the transport of ~220 proteins and several mRNAs and is the sole nuclear exporter of the major tumor suppressor and growth regulatory proteins p53, p73, FOXO, IκB/NF-κB, Rb, p21, and NPM. Our recent findings demonstrate that novel irreversible inhibitors of XPO1, termed Selective Inhibitors of Nuclear Export, or SINE, induce rapid apoptosis in 12 AML and 14 T-ALL cell lines with IC50s of 15-474 nM. In the SINE-sensitive cell lines, BCL2 overexpression suppresses SINE-induced apoptosis, indicating its intrinsic pathway mediation. Oral administration of clinical XPO1 inhibitor, KPT-330, at 15 or 25 mg/kg, induced remarkable growth suppression in MV4-11 human AML cells and MOLT-4 human T-ALL cells engrafted into immunodeficient NSG mice with minimal toxicity to normal mouse hematopoietic cells after 35 days of treatment. Bone marrow biopsies of KPT-330-treated mice were remarkable in that they showed normal hematopoietic cell morphology and cellularity after 35 days of treatment. Significant survival benefit was observed in mice treated with KPT-330, compared to vehicle-treated mice. Recently, we have established primagraft models of AML, using leukemia blasts isolated from patients at diagnosis and engrafted into immunocompromised mice, and demonstrated that KPT-330 exhibits striking anti-leukemic activity against different subtypes of primary AML. The preliminary results of our re-population assays also indicate that KPT-330 specifically targets leukemia-initiating cells (LICs). These findings demonstrate that KPT-330 represents a new "first-in-class" targeted therapy for the treatment of AML and T-ALL that spares hematological toxicity. KPT-330 is now in Phase I clinical trial in patients with AML and other hematological malignancies (NCT01607892).

Poster Board Number: I02

FROM INDUCED PLURIPOTENT STEM CELLS TO HEMATOPOIETIC PROGENITORS: TOWARDS A CELLULAR THERAPY APPROACH FOR INFANTILE MALIGNANT OSTEOPETROSIS

Muggeo, Sharon¹, Neri, Tui¹, Caldana, Maria Elena¹, Focarelli, Maria Luisa², Paulis, Marianna², Strina, Dario², Crisafulli, Laura¹, Scaramuzza, Samantha¹, Lombardo, Angelo³, Naldini, Luigi³, Vezzoni, Paolo², Ficari, Francesca², Villa, Anna²

¹Humanitas Clinical and Research Center, Rozzano (MI), Italy, ²Milan Unit, Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche, Milan, Italy, ³San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Milan, Italy

The induced pluripotent stem cells (iPSc) entrance in the stem cell landscape has given the scientific community a novel approach for

studying human diseases and a new promising tool for regenerative medicine. iPSc generation from patients affected by genetic diseases could allow their site-specific genetic correction followed by differentiation and autologous transplantation for disease cure. Infantile malignant osteopetrosis is a life-threatening recessive bone disease caused by a mutation in the TCIRG1 gene, which severely affects osteoclasts resorbing activity. The resulting increased bone density causes severe growth retardation, thickened bones, and reduced medullary cavity, symptoms recapitulated by the oc/oc mouse. Hematopoietic stem cell (HSC) transplantation is the unique possible treatment, however the chance of cure is strongly limited by the need for a matched donor. Therefore, patients should benefit from the generation of corrected autologous HSCs for a novel approach to therapy. The aim of our project is to generate iPSc from murine wt and affected fibroblasts, to correct the Tcigr1 genetic mutation, to differentiate iPSc into the hematopoietic lineage including HSCs, and to transplant them in vivo to revert the oc/oc phenotype. To generate iPSc lines, as delivery system for the reprogramming genes Oct4, Sox2 and Klf4 we employed a third generation polycistronic lentiviral vector, excisable from the host genome by the Cre recombinase. After reprogramming, iPSc clones with low vector copy number and normal karyotype were chosen, treated with Cre recombinase and sub-cloned to select lines without integrated vectors. Pluripotency of the obtained iPSc was tested by teratoma formation assay, embryonic germ layers in vitro differentiation, and expression of stemness markers through immunocytochemistry and real time PCR. Karyotype analyses showed the presence of normal sets of chromosomes. Importantly, iPSc were successfully derived from oc/oc fibroblasts, and subsequently corrected through homologous recombination upon transfection with a BAC containing wt Tcigr1. iPSc generated from wt and oc/oc fibroblasts were forced to differentiate towards the hematopoietic lineage in a 12 days in vitro protocol in the presence of specific cytokines in order to obtain transplantable cells, without additional transgene over-expression. All tested iPSc successfully differentiated into hematopoietic cells belonging to different lineages, including early progenitors showing a Lineage cKit⁺Sca1⁺ immunophenotype. Multicolour FACS analysis performed over time revealed a differentiation kinetics resembling a physiologic fetal hematopoiesis, with cells expressing the CD41 early hematopoietic marker gradually giving rise to CD45 expressing cells, which comprised mature myeloid cells as well as high proliferative potential colony-forming cells, including mixed-type colonies. Most importantly, we obtained differentiation towards osteoclasts, the relevant cells in our model. Transplants into oc/oc recipients are ongoing. In conclusion, with our studies we will provide a proof of principle for the future clinical use of a new tool to treat osteopetrosis and potentially other genetic blood disorders.

Poster Board Number: I03

THE EXPRESSION OF SOX17 IDENTIFIES AND REGULATES HEMOGENIC ENDOTHELIUM

Clarke, Raedun L.¹, Yzaguirre, Amanda D.², Robitaille, Aaron³, Yashiro-Ohtani, Yumi², Bondue, Antoine⁴, Blanpain, Cedric⁴, Pear, Warren², Moon, Randall³, Speck, Nancy², Keller, Gordon¹

¹McEwen Centre for Regenerative Medicine, Toronto, ON, Canada, ²Abramson Family Cancer Research Institute, University of Pennsylvania, Philadelphia, PA, USA, ³Department of Pharmacology, University of Washington School of Medicine, Seattle, WA, USA, ⁴IRIBHM, Université Libre de Bruxelles, Brussels, Belgium

During embryonic hematopoietic development emerging hematopoietic stem cells (HSCs) are first detected in the major arterial vessels, the most well characterized site being the intra-embryonic region comprising the developing aorta, gonads and mesonephros

(AGM). Within the aorta HSCs arise from a specified type of endothelium, hemogenic endothelium, that undergoes an endothelial to hematopoietic transition resulting in the generation of intra-aortic clusters. Several studies have begun to map the transcriptional regulators of the generation of hemogenic endothelium (HoxA3) and the endothelial to hematopoietic transition (Notch1, Runx1), however the integration of these factors into a cohesive mechanism has not been resolved. Here we identify Sox17 as a key regulator of hemogenic endothelial development. Analysis of Sox17-GFP reporter mice revealed that Sox17 is expressed in hemogenic endothelium and emerging HSCs and that it is required for HSC development. Using the mouse embryonic stem cell differentiation model, we show that Sox17 is also expressed in hemogenic endothelium generated in vitro and that it plays a pivotal role in the development of hemogenic endothelium through the Notch signaling pathway and functions downstream of HoxA3. Our current studies are focusing on defining the signaling pathways that regulate Sox17 expression, the transcriptional co-regulators required for Sox17 function and additional transcriptional targets of Sox17 with emphasis on the canonical Wnt signaling cascade. Taken together, these findings position Sox17 as a key regulator of hemogenic endothelial and hematopoietic development.

Poster Board Number:104

HEMATOPOIETIC STEM CELLS FROM TRANSLSION SYNTHESIS DEFICIENT MICE ARE DEFICIENT IN VITRO AND IN VIVO

van Os, Ronald¹, Tsaalbi-Shtylik, Anastasia², Dethmers-Ausema, Albertina¹, de Wind, Niels², de Haan, Gerald¹

¹Ageing Biology and Stem Cells, University Medical Center Groningen, Groningen, Netherlands, ²Department of Toxicogenetics, Leiden University Medical Center, Leiden, Netherlands

Any damage to the DNA of stem cells that can be transmitted to daughter cells has major consequences for the function of a tissue. Hematopoietic stem cells (HSC) are primitive blood cells that are capable of self-renewal and differentiation into all blood lineages. It has been shown that deficiencies in DNA repair results in accelerated aging (Rossi et al, Nature, 2007). Translesion synthesis is not involved in DNA repair but recruits specialized polymerases, among which are Rev1 and Rev3, capable of bypassing DNA lesions that stall processive DNA replication.

In this study, we tested the hematopoietic stem cell (HSC) frequency in mice, deficient in translesion synthesis polymerases Rev1. Whereas, Rev3 deficient mice are embryonically lethal, Rev1-deficient mice are viable and have a similar lifespan as their wild-type littermates. Rev1-deficient bone marrow was reduced in stem cell numbers at 4 months of age, but stem cell numbers as measured by LinSca-1⁺c-kit⁺ (LSK) and LSKCD48⁺CD150⁺CD34⁺EPCR⁺ phenotyping, were increased but not different from wild-type littermates at 18 months of age. In vitro activity in cobblestone area forming cell (CAFC) assays showed a reduced functionality only in young mice. In old mice, CAFC activity was similar. Both young and old Rev1 deficient mice have a reduced proliferative capacity in vitro in response to growth factors. However, in both cases transplantation of Rev1 bone marrow stem cells showed a severe competitive disadvantage compared to wild type cells. These data are compatible with the mild progeroid phenotype of Rev1 mice (manuscript in preparation). In addition, the data suggests that translesion synthesis deficiency causes enhanced proliferative stress on HSC which may contribute to accelerated aging of the hematopoietic system. In conclusion, hematopoiesis in mice is affected by defects in translesion synthesis, leading to reduced stem cell numbers and compromised functionality that results in aging-like phenotypes.

Poster Board Number:105

THE FUNCTIONAL CHARACTERIZATION OF MIR-125 FAMILY IN HEMATOPOIESIS

Wojtowicz, Edyta, Walasek, Marta Anna, Weersing, Ellen, Broekhuis, Mathilde, Ritsema, Martha, Ausema, Albertina, Bystrykh, Leonid, de Haan, Gerald

Aging and Stem Cell Biology, ERIBA/UMCG, Groningen, Netherlands

MicroRNAs (miRs) are small (22-25 nt), non-coding RNAs, which play an essential role in the control of hematopoiesis. MiRs tune the expression of certain proteins by pairing with the complementary sequence in 3' UTR of the target mRNA. The extensive pairing is attributed to a short, highly conservative sequence called the seed sequence, which in turn is considered as the main determinant of miRNA target repertoire. Furthermore, the seed sequence is the main criterion for miRNAs classification into families. Previously we have identified and validated miR-125a as an important regulator of hematopoietic stem cells (HSCs). Overexpression of miR-125a resulted in increased proliferation in vitro and conferred a competitive advantage to hematopoietic stem/progenitor cells (HSPCs) in vivo (Gerrits et al., 2012). MiR-125a is a member of the miR-125 family (consisting of: 125a, 125b1 and 125b2), which shares more than 85% sequence similarity. Despite of sharing the same seed sequence, there have been accumulated discrepancies in the literature, suggesting that miR-125 family members may have distinct effects on hematopoiesis.

To functionally characterize and compare the phenotypes of miR-125 family members in HSPCs, we overexpressed them in bone marrow cells and functionally assessed these cells in a range of in vitro and in vivo assays. The colony forming unit-granulocyte monocyte assay (CFU-GM) showed that cells overexpressing miRs-125 family members generate more colonies and have increased self-renewal potential as measured by replating activity compared to the control. These findings are consistent with increased and prolonged activity in stem cell surrogate CAFC assay, indicating higher frequency of HSPCs or increased resistance to apoptosis. To further study both scenarios, the data from in vivo transplantation experiments showed decreased apoptosis in the lineage - cell population and a vast proliferative advantage of cells overexpressing miR-125 family members. However, the frequency of phenotypically defined HSCs (Lin-Sca+c-Kit+CD48-150+) was already significantly diminished 10 weeks post transplantation and these cells were absent 7 months post transplantation. At the same time we observed increased numbers of progenitors (CMP/GMP) and differentiation biased towards myeloid lineage. All miR-125 family members showed the same phenotype in vitro and in vivo with slight differences in the strength of the effect. Combined, our data show that all miR-125 family members do indeed modulate HSPCs characteristics in a similar way, enhancing differentiation into myeloid lineage, and expansion of progenitors, while at the same time decreasing the frequency of apoptotic cells in lineage - compartment. To investigate the molecular mechanism of the observed phenotype we are in the process of targets identification of miR-125.

Poster Board Number:106

HUMAN CORD BLOOD HEMATOPOIETIC STEM CELLS AND MULTIPOTENT PROGENITORS RESIDE IN DISTINCT MOLECULAR QUIESCENT STATES

Laurenti, Elisa¹, Xie, Stephanie¹, Frelin, Catherine¹, Doulatov, Sergei², Neumann, Andrea¹, Plumb, Ian¹, April, Craig³, Iscove, Norman¹, Fan, Jian-Bing³, Dick, John E.¹

¹University Health Network, Toronto, ON, Canada, ²Children's Hospital, Boston, MA, USA, ³Illumina inc., San Diego, CA, USA

Hematopoietic stem cells (HSC) have the unique capacity to provide life-long production of all blood cell types, by maintaining a delicate balance between their self-renewal and differentiation capacities. A large body of work in murine models has demonstrated that HSC are mostly quiescent but start proliferating upon activation by a variety

of stimuli, in order to respond to the shifting needs of hematopoietic demand. It is hypothesized that quiescence, by limiting cell cycle activity is crucial for HSC life-long function. However the molecular circuits underlying maintenance and exit from quiescence in homeostatic as well as in stress situations are poorly characterized especially in human HSC. We recently reported that CD49f is a specific human HSC marker; as single Lin⁻ CD34⁺ CD38⁻ CD45RA⁻ CD90⁺ CD49f⁺ cells isolated from cord blood (CB) efficiently generate long-term multilineage grafts in xenograft models. Loss of CD49f expression marks the first step of HSC differentiation, with progression to transiently engrafting multipotent progenitors (MPP), with limited self-renewal potential. These findings provide the unprecedented opportunity to investigate the functional properties and the molecular networks of the most primitive human HSC and its direct progeny. Here we show that both HSC and MPP are maintained in a fully quiescent state (100% G0) in cord blood, and study their kinetics of activation *in vitro* and *in vivo*. To understand how proliferation and reconstitution capacity are integrated at the molecular level, we monitored cell cycle status, doubling times, repopulation capacity and gene expression profiles of HSC and MPP over the course of 32 weeks upon transplantation in NSG mice. In this xenograft model, HSC and MPP underwent an expansion phase during which the majority of cells exited G0 and actively proliferated, then gradually and synchronously regained quiescence over time reaching equilibrium. However, even when subjected to proliferative stress, HSC constantly maintained longer doubling times than MPP, suggesting longer cell cycle duration as a defining property of stemness. Finally, when activated by cytokines *in vitro*, quiescent CB HSC exited G0 later than quiescent CB MPP. These results indicate different kinetics of activation of HSC and MPP.

To understand the molecular basis for the delayed activation of HSC, we tracked dynamic changes in transcription of fully quiescent (CB), activated (expansion phase) and homeostatic (equilibrium phase) HSC and MPP. We used time-series specific algorithms to identify genes that are differentially expressed by HSC and MPP at all stages of the transplantation process, independently of changes in the environment. By this approach, we found 3 core pathways differentially used in HSC and MPP: metabolism, inflammatory response and cell cycle. Most master regulators of cell cycle checkpoints were differentially expressed at the mRNA level (i.e. CDC2...), which resulted in differential protein expression and activity, as demonstrated by functional assays with small molecule compounds and genetic overexpression. Overall, our data demonstrate that circulating human HSC and MPP are fully arrested in G0 but respond differently to the same activating signals, with MPP intrinsically primed for faster entry into cell cycle. In conclusion, human CB HSC and MPP display distinct molecular states of quiescence, which has important consequences for the development of HSC transplantation and expansion protocols.

Poster Board Number: I07

MOLECULAR CHARACTERIZATION OF DORMANCY IN HSCs

von Paleske, Lisa, Tesio, Melania, Trumpp, Andreas
Division of Stem Cells and Cancer, German Cancer Research Center (DKFZ), Heidelberg, Germany

Adult stem cells are required to maintain highly regenerative tissues such as the skin, the intestinal epithelium and the hematopoietic system. Mouse hematopoietic stem cells (HSCs) are the most well characterized somatic stem cells to date. Recently a population of dormant HSCs has been identified within the LSK CD150⁺CD48⁻CD135⁻CD34⁻ population by label retaining assays. Computational modelling suggested a proliferation frequency during homeostasis of about 5 divisions per lifetime. While these dormant HSCs form a silent reservoir of the most potent HSCs during homeostasis, they are efficiently activated to self-renew in response to bone marrow injury. This allows the fast production of progenitors and mature cells ensuring repair and survival. After re-establishment of homeostasis,

activated HSCs return to dormancy, suggesting that HSCs are not stochastically entering the cell cycle but reversibly switch from dormancy to self-renewal under conditions of hematopoietic stress.

To identify the molecular mechanisms underlying dormancy we have compared the mRNA expression profile of dormant and active HSCs within the LSKCD150⁺CD48⁻CD34⁻ population using the H2B-GFP label retaining assay. The analysis revealed differential expression of approximately two hundred genes. In particular, genes promoting proliferation and transcripts encoding a variety of histone proteins were up-regulated in active HSCs. Strikingly, a set of genes involved in megakaryopoiesis and platelet function were up-regulated in these cells. In contrast, dormant HSCs are characterized by high expression of transcripts involved in cell cycle inhibition and inhibition of apoptosis as well as transcripts of proteins involved in cell adhesion.

Poster Board Number: I08

EXPANSION AND ORDERLY DIFFERENTIATION OF MEGAKARYOCYTIC PROGENITOR CELLS GENERATED FROM CORD BLOOD HEMATOPOIETIC STEM/PROGENITOR CELLS AND ITS PHASE I/II CLINICAL TRIALS

Pei, Xuetao, Xi, Jiafei, Liu, Daqing, Zhu, Honghu
Institute of Transfusion Medicine, AMMS, 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 malignancies. 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.

Poster Board Number: I09

PERTURBATION OF GPSM2 (LGN) ENHANCES HEMATOPOIETIC STEM CELL FUNCTION

Ting, Stephen B.¹, Lee, Nicole², Li, Ming², Russell, Sarah³, Godde, Nathan⁴, Humbert, Patrick⁴, Matsuzaki, Fumio⁵, Deneault, Eric⁶, Sauvageau, Guy⁶

¹Haematology, Australian Centre for Blood Diseases, Monash University & Alfred Health, Victoria, Australia, ²Australian Centre for Blood Diseases, Monash University, Victoria, Australia, ³Immune Signalling Laboratory, Cancer Immunology, Peter MacCallum Cancer Centre, Victoria, Australia, ⁴Cell Cycle and Cancer Genetics Laboratory, Peter MacCallum Cancer Centre, Victoria, Australia, ⁵Laboratory for Cell Asymmetry, RIKEN, Center for Developmental Biology, Kobe, Japan, ⁶Molecular Genetics of Stem Cells, Institute for Research in Immunology and Cancer, Montreal, QC, Canada

A gain of function screen of candidate cell polarity genes identified the gene, Gpsm2 (G protein signaling modulator 2) also known as LGN or Pins, as an enhancer of haematopoietic stem cell (HSC) function.

In multiple independent experiments, Gpsm2-transduced HSCs and cultured for 7 days in vitro compared to vector-transduced HSCs showed enhanced HSC repopulation out to 20 weeks-plus post transplant. At these time points, bone marrow and thymic DNA from multiple recipient mice were analysed via Southern blots with a GFP probe. This showed Gpsm2-transduced HSCs retained multipotency but that only one out of a minimum of 12 clones had undergone in vitro symmetrical expansion. A (current) limiting dilution assay will assess quantitatively this relative absence of HSC expansion. Although, in vitro assays with Gpsm2-transduced bone marrow cells showed reduced colony formation and slightly attenuated cell proliferation, limiting dilution assays in secondary transplants showed Gpsm2-transduced HSCs maintained self-renewal ability together with a significant ($p=0.009$) 4-fold increase in HSC frequency compared to vector-transduced HSCs. This presumably reflects the importance of the interaction between Gpsm2-transduced HSCs within the niche in vivo. Mammalian Gpsm2 comprises an N-terminal domain composed of ten Leucine-Glycine-Asparagine (LGN) repeats within seven to eight tetratricopeptide motifs, which functions to bind NuMA (Nuclear Mitotic Apparatus) and a C-terminal domain containing four GoLoco repeats that modulates guanine nucleotide exchange via G α i interaction. In effect, Gpsm2 is an integral component that coordinates G protein signalling to mitotic spindle orientation during cell division. A mouse mutant lacking all GoLoco repeats of the C-terminal domain (LGN Δ C) is viable, where analyses of the neuroprogenitors of this mouse revealed altered asymmetrical versus symmetrical cell divisions without a detrimental effect on neuronal production (Konno et al. Nat Cell Biol 2008). A subsequent study of the role of LGN in epidermal development identified that a significant (>80%) knockdown of LGN via shRNA also altered asymmetrical cell divisions of epidermal stem-progenitor cells, however with a functional loss of skin barrier (Williams et al. Nature 2011). This defect could be partially rescued by the LGN N-terminal protein suggesting the LGN Δ C mouse still has functional, albeit, incomplete LGN/Gpsm2 function. We have analysed haematopoiesis in the LGN Δ C mouse. There were no significant differences in wild-type versus homozygous LGN Δ C LSK numbers, in vitro colony formation or in vivo CFU-spleen assays. However, limiting dilution assays showed a 7-fold increase ($p=0.02$) in HSC frequency in the LGN Δ C mouse, and competitive CD45.2/CD45.1 transplantations analysed at 16 weeks post transplant showed CD45.2 LGN Δ C HSCs reconstituted significantly ($p=0.015$) better compared to CD45.2 wild-type HSCs. On the premise that Gpsm2 function of controlling stem cell division-fate is conserved, we are in the process of analysing HSC asymmetric divisions-fate in the contexts of LGN/Gpsm2 Δ C and Gpsm2 gain and loss of function as a potential mechanism of how perturbation of Gpsm2 enhances HSC function.

Poster Board Number: 110

IMPROVED MANIPULATION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS (HSPC) FOR EX VIVO GENE THERAPY

Zonari, Erika, Boccalatte, Francesco, Plati, Tiziana, Escobar, Giulia, Ranghetti, Anna, Gentner, Bernhard, Naldini, Luigi
San Raffaele Telethon Institute for Gene Therapy, Milan, Italy

The latest lentiviral-based HSPC gene therapy trials have demonstrated their therapeutic potential in curing genetic diseases. We here set out to optimize different steps of ex vivo HSPC manipulation. First, we tested the dynamic change of surface markers on CD34+ HSPC from cord blood (CB) and bone marrow (BM) during in vitro culture in serum-free maintenance conditions (TSF6: SCF, TPO, FLT3L, IL6, all 50ng/ml). While positive HSPC markers, such as CD34, CD133 and CD90, were detectable on a progressively decreasing fraction of cells during the culture, negative HSPC markers, such as CD38, rapidly lost their informative value. After 1 and 2 weeks, 0.97+0.12% and 0.56+0.06% of CB cells were CD34hiCD90+, respectively. Strikingly, >90% of NSG mouse repopulating capacity was contained in the CD34hiCD90+ fraction of BM and CB at 1 and 2 weeks of culture,

respectively, as shown by competitive repopulation assays. By adding an arylhydrocarbon receptor antagonist (SRI) to the culture, we could significantly increase the number of CD34hiCD90+ cells in CB and BM during a 2-week culture, supporting its use when extended culture times are contemplated, such as for genome editing protocols. Next, we added dmPGE2 to the TSF6/SRI culture cocktail at the time of thawing. dmPGE2 increased the recovery of vital CD34+ cell by 25-50%. Furthermore, we noted a 30-60% increase in LV transduction efficiency, which was maintained long term in vivo, after transplantation into NSG mice. Considering also the published data on the beneficial effect of dmPGE2 on engraftment, which was not specifically addressed in our experimental design, the use of dmPGE2 represents a straightforward means to boost the efficacy of HSPC in vitro manipulation, e.g. in the framework of clinical gene therapy protocols. Several advantages are associated to the use of more highly enriched HSC populations for gene therapy, including the downscaling of culture volumes and vector dose, the better maintenance of stem cell properties in pure HSC cultures due to the reduction of inhibitory feedback signaling arising from spontaneously differentiating cells, and the reduced integration load infused into the patient. We are thus exploring innovative selection strategies to obtain HSPC populations with a 5-10 fold higher long-term engrafting cell content respect to the CD34+ cell fraction currently used as the standard for HSC gene therapy protocols. We are currently modeling the genetic modification and transplantation of highly purified HSC subsets obtained from CB and adult human HSPC sources such as BM and mobilized peripheral blood by xenotransplantation into NSG mice. In summary, the proposed protocol modifications could substantially improve the efficacy, safety and feasibility of future ex vivo gene therapy studies.

Poster Board Number: 111

A MECHANISTIC ROLE FOR MIR-126, A HEMATOPOIETIC STEM CELL MICRORNA, IN ACUTE LEUKEMIAS

Nucera, Silvia¹, Giustacchini, Alice¹, Fanciullo, Cristiana¹, Boccalatte, Francesco E.¹, Plati, Tiziana¹, Vago, Luca², Bernardi, Massimo², Lechman, Eric³, Dick, John E.³, Naldini, Luigi¹, Gentner, Bernhard¹
¹San Raffaele Telethon Institute for Gene Therapy, Milan, Italy, ²San Raffaele Scientific Institute, Milan, Italy, ³University Health Network, Toronto, ON, Canada

We have recently shown that miR-126 expression faithfully identifies the engrafting fraction of bone marrow (BM) and cord blood (Gentner et al, Sci Transl Med 2010), in which it regulates hematopoietic stem cell (HSC) pool size by modulating cell cycle progression through interactions with the PI3K/AKT signaling pathway (Lechman et al., Cell Stem Cell 2012). Acute myeloid leukemia (AML) is hierarchically organized and may be sustained by leukemic stem cells (LSC). miRNA profiling of functionally validated AML subfractions (Lechman et al, unpublished) has shown a significant enrichment of miR-126 in LSC. We have exploited a lentiviral miR-126 reporter vector in 11 primary AML cases (4 AML with CBF, core binding factor; mutations; 7 AML with normal karyotype) and followed miR-126 activity in these AML with single cell resolution after xenotransplantation into NSG mice. We found variable miR-126 activity, which correlated well with miRNA expression measured by quantitative PCR in the different diseases, with highest expression in CBF-mutated AML. Interestingly, across all 11 studied AML cases, there was substantial heterogeneity in terms of miR-126 expression in subpopulations of each single disease. Significantly higher miR-126 levels were found in CD34+, and particularly in CD34+CD38- AML cells, a fraction that is enriched for LSC in most cases of AML (Eppert et al., 2011). LSC are supposed to be resistant to chemotherapy. To see whether there is a correlation between miR-126 expression and disease progression, we measured miR-126 levels in sorted blasts from paired diagnosis/relapse (n=5) and diagnosis/chemotherapy-refractory (n=8) patient samples. Strikingly, we found a significant up-regulation of miR-126 in the disease progression sample, suggesting a mechanistic role for miR-126 in

chemotherapy resistance in a most common clinical context. We expanded our studies to acute lymphoblastic leukemia (ALL), also in light of our recent data that miR-126 overexpression in normal mouse BM leads to the development of moderately penetrant, high-grade lymphoid neoplasms (Giustacchini et al., unpublished data). Expression of miR-126 was characterized in 12 primary adult ALL patient samples (7 had the Philadelphia translocation) by quantitative PCR and by miR-126 reporter after transplantation into NSG mice. miR-126 expression was found to be high in most ALL cases, often surpassing the levels found in normal HSC. Interestingly, unlike in normal HSC or in AML, miR-126 expression was independent from expression of its host gene, EGFL7, suggesting an ALL-specific regulation of the miR-126 locus. In 7 out of 12 ALL cases, we identified distinct subpopulations exhibiting different levels of miR-126 activity, and miR-126 (hi) ALL cells were more frequently contained in the CD34+ cell fraction. Functional studies are under way to define the mechanistic role of miR-126 in adult ALL and the biological implications of its heterogeneous expression in ALL subpopulations.

Poster Board Number: I 12

HIF2A PROTECTS HUMAN HEMATOPOIETIC STEM/PROGENITORS AND ACUTE MYELOID LEUKEMIA CELLS FROM APOPTOSIS INDUCED BY ENDOPLASMIC STRESS

Rouault-Pierre, Kevin¹, Lopez Onieva, Lourdes¹, Foster, Katie¹, Anjos-Afonso, Fernando¹, Lamrissi-Garcia, Isabelle², Serrani-Sanchez, Martin², Ivanovic, Zoran³, de Verneuil, Hubert², Gribben, John⁴, Taussig, David⁴, Reza Rezvani, Hamid², Mazurier, Frédéric², Bonnet, Dominique¹
¹Cancer Research UK, London, United Kingdom, ²INSERM U 1035, Université Bordeaux Segalen, Bordeaux, F-33076 France, Bordeaux, France, ³Université Bordeaux Segalen, Bordeaux, F-33076 France, Établissement Français du Sang-Aquitaine Limousin, Bordeaux, F-33000, France, CNRS UMR 5164, Bordeaux, F-33076 France, Bordeaux, France, ⁴Department of Haemato-Oncology, Barts Cancer Institute, Queen Mary University of London, London, EC1M 6BQ, United Kingdom, London, United Kingdom

Hematopoietic stem cells (HSCs), which reside in bone marrow niches, are exposed to low levels of oxygen and follow an oxygen gradient throughout their differentiation. Hypoxia-inducible factors (HIFs) are the main factors regulating the cell response to oxygen variation. Recent studies using conditional knockout mouse models have unveiled a major role of HIF-1 α in the maintenance of murine HSCs, however the role of HIF-2 α is still unclear. Here, we show that knockdown of HIF-2 α , and to a much lower extent, HIF-1 α impedes the long-term repopulating ability of human CD34+ umbilical cord blood derived cells. The defects observed in hematopoietic stem and progenitor cell (HSPC) function after HIF-2 α knockdown was due to an increase in the production of reactive oxygen species (ROS), which increases the endoplasmic reticulum (ER) stress in HSPCs and triggers apoptosis by the activation of the unfolded-protein-response (UPR) pathway. Importantly, HIF-2 α deregulation also resulted in a significant decrease of engraftment of human acute myeloid leukemia (AML) cells. Overall, our data demonstrates a key role of HIF-2 α in the maintenance of human HSPCs and in the survival of primary AML cells.

Poster Board Number: I 13

DO WE HAVE A WORKABLE CLINICAL PROTOCOL TO OBTAIN LYMPHO-HEMATOPOIETIC STEM CELLS FROM EMBRYONIC STEM CELLS OR INDUCIBLE PLURIPOTENT STEM CELLS IN CULTURE?

Chen, Una

ISPI E.V. International Senior Professional Institute, Wuerzburg, Germany

Lympho-hematopoietic stem cells (L-HSC) are defined as the hematopoietic stem cells possessing the potential to differentiate into mature lymphoid cells and other blood cells, in vivo, and possibly in culture. The source of L-HSC after birth could be bone marrow (BM)

and cord blood (CB) whereas before birth, it could be yolk sac, aorta-gonad-mesonephros, and fetal liver. In the earliest developmental stage, it could be embryonic stem cells (ESC) and the inducible pluripotent stem cells (iPSC). Transplantation of L-HSC from BM and CB to treat lymphoid-cell-related diseases is part of clinical practice worldwide. But the immune complications that aroused, mainly histocompatibility-related, led investigators to propose the use of cells derived before birth to enhance the chance of inducing tolerance. Given that the sources of fetal stem cells are extremely limited due to their rareness and ethical considerations increasing attention and efforts are focusing on deriving L-HSC from human-embryonic stem cells (ESC) and/or induced pluripotent stem cells (iPSC).

The clinical application of these potential cells is currently in debate. The efficient generation of L-HSC has been difficult until today. Various technical and logistical hurdles need to be solved before reaching the generation of transplantable human L-HSC from ESC/iPSC. In addition, whether L-HSC derived from such sources would raise fewer complications than those from BM and CB is also an open question. It remains a challenge to investigate whether L-HSC derived from ESC/iPSC are indeed immune privileged in syngenic transplantation setting. Differentiation of these cells does express major histocompatibility antigens and thus increase their immunogenicity. In a combined in vitro and in vivo assay system, thymic education by donor-derived thymic environment and tolerance to both donor and recipient has been demonstrated. The transplantation experiment using L-HSC from unmodified mouse (m) ESC to syngenic immune-competent mice could not be performed due to the lack of markers to distinguish donor vs host lymphocytes. MESC clones containing transgenic-marker did not allow the differentiation of mESC to the culture stage where blood islands could be detected thus; no further experimentation was performed and a similar approach using mouse iPSC has not been reported yet. Clinical translation can only be successful if good knowledge of biological-immunological processes linked to their therapeutic effect exists.

Today, it is mandatory to discuss the logistics, technical and immunologic issues within the context of transplantation to provide realistic insights into the applicability of these cells in clinical settings. Analyzing and sharing such information, experience, and resources could derive promising guidelines and clinical protocols for "therapeutic lympho-hematopoietic cells".

Poster Board Number: I 14

PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS AS A HUMAN-DERIVED MODEL TO STUDY METACHROMATIC AND GLOBOID CELL LEUKODYSTROPHY

Frati, Giacomo¹, Meneghini, Vasco¹, Cavazzin, Chiara¹, De Cicco, Silvia¹, Lombardo, Angelo¹, Paulis, Marianna², Morena, Francesco³, Sanvito, Francesca⁴, Villa, Anna², Martino, Sabata³, Naldini, Luigi¹, Gritti, Angela¹
¹San Raffaele Telethon Institute for Gene Therapy (TIGET), San Raffaele Scientific Institute, Milano, Italy, ²Milan Unit, IRGB-CNR, Clinical and Research Center and Humanitas, Rozzano, Italy, ³Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Perugia, Italy, ⁴Anatomy and Histopathology Unit, San Raffaele Scientific Institute, Milano, Italy

Metachromatic and Globoid Cell Leukodystrophies (MLD, GLD) are rare Lysosomal Storage Disorders (LSD) caused by genetic defects in the activity of arylsulfatase A (ARSA) and galactosylceramidase (GALC), respectively, which are key enzymes in the catabolism of myelin-enriched sphingolipids. While primary genetic and biochemical defects are described in these LSD, the paucity of currently available human-derived CNS model systems hampers accurate exploration of downstream events in relevant target cells. The establishment of an in vitro model recapitulating CNS pathogenesis may help understanding the cellular and molecular events underlying disease manifestations. Here we report the derivation of a collection of induced pluripotent stem cell (iPSC) lines generated through somatic reprogramming of fibroblasts obtained from MLD and GLD patients as well as

from normal donors (ND). Bona-fide iPSC clones have been selected based on stable and normal karyotype, expression of pluripotency markers and ability to generate teratomas when injected in immunodeficient mice.

On these cells we checked for the appearance and progression of disease markers, exploring the hypothesis of a pre-symptomatic disease pathogenesis in MLD- and GLD-derived iPSCs. Morphological, biochemical and molecular analyses revealed increased lysosomal storage and impaired lysosomal trafficking in MLD and GLD iPSCs as compared to ND counterparts. Preliminary data on MLD clones revealed that these pathological hallmarks were reduced when (supra) physiological ARSA expression was achieved by lentiviral-mediated gene transfer performed either before (in fibroblasts) or after the reprogramming event (in iPSCs). Our data indicate that a more robust and stable ARSA expression is achieved by post-reprogramming gene transfer. By applying specific culture conditions we differentiated disease-bearing and gene-corrected iPSCs into neural stem/progenitor-like cells (NPCs) that are further differentiating into neurons and glial cells, with a particular focus on oligodendrocytes, the most affected cell type in MLD and GLD. We are currently assessing the functional features of iPSC-derived NPCs and differentiated progeny, testing the efficacy of gene transfer in providing stable and sufficient levels of the functional GALC and ARSA enzyme to revert a potential global disease phenotype. These studies pave the way for a comprehensive modelling of the degenerative mechanisms underlying MLD and GLD pathology. Also, combining iPSC technology (to generate patient-specific iPSC-derived neural cells) with efficient gene replacement platforms (to correct the genetic defect) is instrumental to obtain homogeneous and renewable sources of disease-free autologous human neural cells that could be considered for development of clinical protocols based on autologous cell transplantation for a variety of neurodegenerative monogenic diseases, after careful validation of their safety and efficacy in relevant disease animal models.

Poster Board Number: I 15

A PHASE II CLINICAL TRIAL OF G-CSF MOBILIZED CD34+ CELL THERAPY TO EXPLORE ENDPOINT SELECTION AND TIMING IN NO OPTION PATIENTS WITH CRITICAL LIMB ISCHEMIA

Fujita, Yasuyuki¹, Kinoshita, Makoto¹, Furukawa, Yutaka², Nagano, Tohru³, Fukushima, Masanori⁴, Nada, Adel⁵, Losordo, Douglas W.⁶, Asahara, Takayuki⁷, Okita, Yutaka⁸, Kawamoto, Atsuhiko¹

¹Division of Vascular Regeneration, Unit of Regenerative Medicine, Institute of Biomedical Research and Innovation, Kobe, Japan, ²Department of Cardiovascular Medicine, Kobe City Medical Center General Hospital, Kobe, Japan, ³Department of Dermatology, Kobe City Medical Center General Hospital, Kobe, Japan, ⁴Translational Research Informatics Center, Kobe, Japan, ⁵Baxter Healthcare Corporation, Deerfield, IL, USA, ⁶Department of Medicine, Division of Cardiology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA, ⁷Department of Regenerative Medicine Science, Tokai University School of Medicine, Isehara, Japan, ⁸Division of Cardiovascular Surgery, Department of Surgery, Kobe University Graduate School of Medicine, Kobe, Japan

Background: A prior phase I/IIa clinical trial provided evidence for safety, feasibility and potential efficacy of intramuscular injection of G-CSF-mobilized CD34+ cells at weeks 4 and 12 post cell therapy in patients with no-option critical limb ischemia (CLI). **Methods:** A phase II trial of CD34+ cell therapy was conducted in patients with CLI to explore endpoint selection and timing. No-option CLI patients (N=11) with atherosclerotic peripheral arterial disease or Buerger's disease underwent intramuscular transplantation of G-CSF-mobilized CD34+ cells isolated by an immuno-magnetic sorting system. The proportion of patients becoming CLI-free, ischemic rest pain scales, and functional parameters of limb perfusion and exercise tolerance were evaluated at weeks 2, 4, 8, 12, 24, 36 and 52 post cell therapy. **Results:** There were no deaths in the study, and 1 patient underwent major amputation through week 52. Overall, the Rutherford scale significantly improved from week 24 (3.0[2.0~5.0]) vs baseline (4.0[4.0~5.0]) (P<0.01). The

CLI-free ratio serially increased and peaked (85.7%) at week 36. Visual Analogue Scale (2.1±2.7 vs 5.4±2.3, P<0.0001), Wong-Baker FACES pain rating scale (1.0[1.0~3.0] vs 3.0[2.0~4.0], P<0.001) and Rest Pain Scale (1.0[1.0~3.0] vs 2.0[2.0~4.0], P<0.05) significantly improved beginning at week 2 vs baseline. Skin perfusion pressure (29.2±6.4 vs 23.8±5.6 mmHg, P<0.05), transcutaneous partial oxygen pressure (35.4±15.4 vs 22.9±15.8 mmHg, P<0.05) and pain-free walking distance (204.4±136.6 vs 93.9±68.4 m, P<0.01) improved from week 2, total walking distance from week 8 (304.1±95.5 vs 204.5±148.4 m, P<0.05) and toe brachial pressure index from week 12 (0.3±0.1 vs 0.2±0.1, P<0.05) vs baseline. These parameters plateaued at week 36 or 52. Serial changes in Rutherford's category correlated with changes in the Rest Pain Scale (P<0.05), but not with any functional parameters. **Conclusions:** In this study, ischemic rest pain scales and physiological parameters improved relatively early after the cell therapy, then plateaued later accompanied by recovery from the CLI state. Rutherford's category and CLI-free ratio at week 36 or later may be suitable endpoints in cell therapy clinical trials for CLI. Functional parameters should be evaluated independently of such clinical endpoints for ischemia severity.

Poster Board Number: I 17

MICRORNA-15A/16 AND B1 CELL DEVELOPMENT IN A MOUSE MODEL OF CHRONIC LYMPHOCYTIC LEUKEMIA

Underbayev, Chingiz¹, Kasar, Siddha¹, Degheid, Heba², Marti, Gerald E.², Lightfoote, Marilyn², Raveche, Elizabeth¹

¹Pathology and Lab Medicine, Rutgers University, Newark, NJ, USA, ²FDA, White Oak, MD, USA

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. Similar to human CLL this mouse model exhibits a point mutation six bases downstream from pre-miR-16 region on chromosome 14. In both NZB mouse model and human CLL, the disease is characterized by the presence of a malignant clone of B-1 cells expressing CD5 and B220 and reduced levels of miR-15a/16. 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. Our in vivo and in vitro studies on NZB iPS and hematopoietic stem cells (HSC) differentiation towards B-cell lineage cells followed by multicolor flow based cell sorting 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 diverges B-cell differentiation towards a higher expression of B220 (CD45R) and IL7Ra suggestive of an enhanced B cells maturation pathway. Our results support the hypothesis that miR-15a plays a role in B1 to B2 lymphocyte development skewing during B cell maturation by regulating the expression of a number of genes such as B220, PU.1 and IL7Ra. This work will help further uncover early B1/B2 cell development defects associated with low levels of miR-15a/16 in the course of CLL.

Poster Board Number: I 18

TOWARDS COMBINED GENE AND CELL THERAPY USING INDUCED PLURIPOTENT STEM CELLS FROM A PATIENT WITH SEVERE CONGENITAL NEUTROPENIA

Hoffmann, Dirk¹, Zychlinski, Daniela¹, Geis, Franziska¹, Kühle, Johannes¹, Klein, Christoph², Baum, Christopher¹, Schambach, Axel¹

¹Experimental Hematology, Hannover Medical School, Hannover, Germany,

²University Children's Hospital, Maximilians University, Munich, Germany

The generation of induced pluripotent stem cells (iPSCs) from patient-specific somatic cells provides a renewable source for autologous cells and plays a promising role in regenerative medicine in future. The combination of iPSC technology and gene therapy opens the opportunity to treat rare congenital immunodeficiencies and to

minimize genotoxicity of retroviral vectors by selecting clones with the safest genomic insertions. Thus, in future therapies genetically-modified iPSC-derived hematopoietic progeny with a clonal identity may be used for autologous transplantations to avoid the adverse events observed during hematopoietic stem cell gene therapies linked to insertional activation of proto-oncogenes.

We reprogrammed fibroblasts derived from dermal biopsies of a severe congenital neutropenia (SCN) patient with a nonsense-mutation in the glucose-6-phosphatase (G6PC3) gene. To achieve efficient reprogramming with low inserted vector copies, we developed a lentiviral polycistronic vector expressing the Yamanaka factors Oct4, Sox2, Klf4, cMyc and a red fluorescence protein. Subsequently, the reprogramming cassette flanked by two Flp recombinase targeting sites could be efficiently excised in the iPSC clones using the technique of retroviral protein transfer for transient delivery of Flp-recombinase. The generated iPSC clones exhibited the capacity to differentiate on OP9 stromal cells to hematopoietic cells of the myeloid lineage. Reduced numbers of mature neutrophils were generated in vitro from SCN patient iPSCs compared to control cells. Interestingly, this reflects the phenotype in the patients, who show the typical paucity of mature granulocytes in the bone marrow caused by an increased susceptibility to apoptosis. For the functional correction of the G6PC3 gene and optimal transgene expression in human iPSCs we tested a set of self-inactivating retro- and lentiviral vectors and different promoter configurations. We generated a lentiviral correction vector with a combination of an ubiquitous chromatin opening element and a strong viral promoter to overcome the general problem of low transduction rates and vector silencing in pluripotent stem cells.

In summary, we came up with a promising iPSC-derived in vitro disease model for the G6PC3 disorder, which will serve as a tool for testing the potency of lentiviral vector-based gene therapy, for performing drug screens and for studying the cellular function of the G6PC3 enzyme during granulopoiesis. Perspective, functionally corrected iPSC-derived myeloid progenitors and mature granulocytes can be transplanted in appropriate mouse models to evaluate the potential of these cells for future clinical applications.

Poster Board Number: I19

THE EPIDERMAL STEM CELL: A NEW APPROACH TO IDENTIFY STEM CELLS AS VIABLE LABEL-RETAINING CELLS

Specker, Elisa, Boukamp, Petra

Genetics of Skin Carcinogenesis, German Cancer Research Center, Heidelberg, Germany

Stem cells in the human interfollicular epidermis are still difficult to identify, mainly due to a lack of definitive surface markers. Since stem cells rarely divide, they can be functionally identified as label retaining cells (LRCs). Until now, the isolation of DNA labelled (e.g. IdU) vital LRCs is restricted due to the need of fixation to visualize the DNA label. With the generation of a lentiviral based vector expressing a Tet-Off controlled histone 2B-GFP (Tet-Off-H2B-GFP) reporter gene, a new tool for the detection and isolation of viable LRCs was established.

The aim of this study is to analyse the regulation of human epidermal stem cells and to identify more reliable surface markers for their unbiased identification. Our first goal was to infect human keratinocytes with the Tet-Off-H2B-GFP vector. These transduced keratinocytes are then cultivated in organotypic cultures (OTCs). OTCs mimic the in vivo human skin and allow long-term growth and differentiation of keratinocytes providing the basis for tissue homeostasis. We could show that the H2B-GFP transduced keratinocytes still have the ability for long-term tissue regeneration (up to 3 month). Furthermore we are able to detect LRCs with pulse/chase experiments in OTCs. To analyse the expression profile and functional competence of these potential stem cells, we will now isolate LRCs. After a chase period of at least 6 weeks the epithelium in our OTCs can be removed and the cells can be dissociated into a single-cell suspension. With this method we are able to isolate mostly LRCs of the basal layer where the stem cells are located. The isolated LRCs will be now isolated and purified via FACS sorting and analysed.

Poster Board Number: I20

REGULATION OF ASYMMETRIC/SYMMETRIC STEM CELL DIVISION IN HUMAN EPIDERMIS

Noeske, Katharina, Boukamp, Petra

German Cancer Research Center, Heidelberg, Germany

The human epidermis is maintained by a balance of stem cell maintenance and proliferation with subsequent differentiation. It was long suggested that the fate of the epidermal stem cell is decided by an asymmetric cell division, with one daughter remaining as a stem cell and the other becoming a transit amplifying cell which proliferates and finally differentiates. This stem cell hierarchy is, however, questioned lately. Clayton and coworkers (Clayton et al. 2007) propose that all basal cells have stem cell function and expand by symmetric or asymmetric division. According to this hypothesis, only basal cells have proliferative potential. Thus, it presently remains elusive which hypothesis is correct and how these different types of division are regulated.

To monitor the mitotic behaviour of epidermal stem cells in an in vivo-like situation we use an advanced long-term organotypic culture model. This allows for stem cells to be labelled with a base analogue (pulse) and to be followed for several weeks (chase). Thereby, the slowly cycling stem cells establish as label-retaining cells (LRCs) in the basal layer and can be investigated for their mode of division by using markers specific for e.g. asymmetric division. Our preliminary results show that mitosis is not restricted to the basal layer but also occurs in suprabasal layers. In addition, we found different angles of division both in basal as well as in suprabasal mitoses.

We therefore propose a stem cell model including a stem cell population and a transit amplifying population with proliferative potential with asymmetric divisions occurring in both populations.

Poster Board Number: I21

KIT+ PROGENITOR CELLS GENERATE THE BRANCHING ARCHITECTURE OF EPITHELIAL ORGANS

Lombaert, Isabelle M.A., Abrams, Shaun R., Hoffman, Matthew P.

National Institutes of Health, Bethesda, MD, USA

Irradiation damage to salivary glands during cancer treatment often leads to a permanent loss of saliva production. Regeneration of irradiated submandibular glands (SMGs) in mice can occur after transplantation of SMG epithelial Kit+ progenitors. However, the mechanism by which Kit+ progenitors regenerate the tissue is not well understood. Using fetal SMG development, we show that signals from the mesenchyme stimulate both Fgfr2b and Kit signaling to expand distal Kit+ progenitors. These progenitors in turn produce neurotrophic factors that promote neuronal innervation, which influences a separate population of proximal progenitors that form ductal structures. Ultimately, reiterative rounds of this multicellular communication establish the branching architecture of the developing organ, and a similar molecular mechanism maintains adult tissue homeostasis. This model of organogenesis provides a template for regenerative medicine and may also have implications for targeting the Kit and Fgfr2b receptor kinases in cancers.

Poster Board Number: I22

COLLECTIVE MOTION DYNAMICS OF HUMAN EPIDERMAL KERATINOCYTE STEM CELLS

Nanba, Daisuke¹, Nanba, Fujio¹, Tate, Sota¹, Imai, Matome¹, Matsushita, Natsuki¹, Toki, Hiroshi², Higashiyama, Shigeki¹, Barrandon, Yann³

¹Ehime University, Toon, Ehime, Japan, ²Osaka University, Ibaraki, Osaka, Japan, ³EPFL & CHUV, Lausanne, Switzerland

Cultured human epidermal keratinocyte stem cells (holoclones) are crucial for regenerative medicine for burns and genetic disorders. In serial culture, holoclones progressively lose their proliferative capacity to become transient amplifying cells with limited growth

(paraclones), a phenomenon termed clonal conversion. Although it negatively impacts the culture lifespan and the success of cell transplantation, little is known on the molecular mechanism underlying clonal conversion. Here, we show that holoclones and paraclones differ in their actin filament organization, with actin bundles distributed radially in holoclones and circumferentially in paraclones. Moreover, actin organization sets the stage for a differing response to epidermal growth factor (EGF), since EGF signaling induces a rapid expansion of colony size in holoclones and a significant reduction in paraclones. Furthermore, inhibition of PI3K or Rac1 in holoclones results in the reorganization of actin filaments in a pattern that is similar to that of paraclones. Importantly, continuous Rac1 inhibition in holoclones results in clonal conversion and reduction of growth potential. We also demonstrate the difference in collective motion dynamics between holoclones and paraclones without any stimuli. Together, our data connect loss of stem cells to collective motion dynamics of keratinocyte colonies.

Poster Board Number: I23

DEREGULATION OF STEM CELLS SIGNALING PATHWAYS IN ORAL SQUAMOUS CELL CARCINOMA STEM CELLS

Rodrigues, Maria Fernanda S.¹, Paiva, Nathália¹, Rodini, Camila O.², Nunes, Fabio D.¹

¹Oral Pathology, University of São Paulo, São Paulo, Brazil, ²Histology, University of São Paulo, Bauru, Brazil

Oral squamous cell carcinoma (OSCC) is the most common cancer of oral cavity, although no improvements on treatment and survival rates have been achieved for the last decade. Recently, it was reported that OSCC, as well as other hematopoietic and solid tumors, presents a subpopulation of tumor-initiating cells that apparently correspond to cancer stem cells (CSC), also responsible for tumor growth and recurrence. This knowledge raised the perspective of new therapeutic approaches targeting this subset of cells as well as the need of a better understanding of tumor development mechanisms. The main purpose of the present study was to investigate which cell signaling pathways are deregulated in the CSC subpopulation sorted from an OSCC cell line based on CD44 cell surface marker expression level. Additionally, we have investigated the expression level of other CSC markers, such as Nanog, CD133 and Oct4, within the CSC and non-CSC subsets of tumor cells. After FACS-sorting SCC4 CD44High (CSC) and CD44Low (non-CSC) subpopulations, cells were plated in culture for 72hrs and total RNA extracted for subsequent analysis of the expression level of Nanog, CD133 and Oct4 by qRT-PCR. In order to assess the differential expression level of several genes related to stem cell signaling in both CD44High and CD44Low sorted cell fractions, we used the commercially available RT2 Profiler™ PCR Array and the relative expression level was determined by the 2- $\Delta\Delta$ Ct method. Results revealed that Nanog, CD133 and OCT4 transcripts were indeed significantly highly expressed in SCC4 CD44High compared to CD44Low subpopulation. More importantly, the wider gene expression screening revealed that genes related to NOTCH, Hedgehog, TGF β and Wnt signaling pathways were at least 5-fold overexpressed in SCC4 CD44High subpopulation when compared to CD44Low cells. We can conclude that sorted CD44High subpopulation corresponds to the CSC subset at the molecular level and, subsequently, important stem cell signaling pathways that are deregulated in oral CSC may contribute to the development, behavior and prognosis of OSCC.

Poster Board Number: I24

CONCATENATED CIRCADIAN OSCILLATIONS TIME HUMAN EPIDERMAL STEM CELL FUNCTIONS

Solanas, Guiomar¹, Janich, Peggy², Toufighi, Kiana¹, Serrano, Luis¹, Lehner, Ben¹, Aznar-Benitah, Salvador¹

¹CRG, Barcelona, Spain, ²Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland

Human skin must cope with harmful environmental factors that are circadian in nature, yet it is not known how circadian rhythms modulate the function of human epidermal stem cells. Here we have combined timed high-throughput transcriptome analysis and functional data to determine how the clock machinery modulates the behavior of human epidermal stem cells and their differentiated counterparts. Intriguingly, our results show that core clock genes peaked in a concatenated manner along the 24 hr of the day, thereby establishing intra-diurnal and intra-nocturnal temporal intervals. Expression profiling indicated that each of these successive clock peaks was associated with a peak in the expression of specific subsets of transcripts that vary between the undifferentiated and differentiating states. Gene ontology analyses suggest that these concatenated transcriptional subsets temporally segregate the predisposition of epidermal stem cells to respond to specific cues, such as those that trigger their proliferation or differentiation. We have functionally shown that the response of human epidermal stem cells to the pro-differentiation cues TGF β and calcium was indeed time-of-the-day dependent. Forced circadian arrhythmia profoundly affected stem cell proliferation and differentiation, both in culture and in stable xenografts in immunocompromised mice. We hypothesize that this intricate mechanism ensures epidermal homeostasis by providing epidermal stem cells with temporal functional cues along the day. The results provide insights to why perturbation of the circadian rhythm may contribute to human epidermal aging and to a predisposition to carcinogenesis.

Poster Board Number: I25

DIRECTED DIFFERENTIATION OF INNER EAR HAIR CELL PROGENITORS FROM MOUSE EMBRYONIC STEM CELLS

Zine, Azel¹, Abboud, Nesrine², Fontbonne, Arnaud², Watabe, Isabelle², Feron, François³

¹Laboratoire des Neurosciences Intégratives & Adaptatives, CNRS UMR 7260, Marseille, France, ²Integratives & Adaptatives Neurosciences, UMR CNRS 7260, Marseille, France, ³Neurobiologie des Interactions Cellulaires et Neuropsychopathologie, CNRS UMR 7259, Marseille, France

The ear develops through the transformation of embryonic ectoderm into the labyrinth and, through a stepwise molecular restriction of cell fate options, into neurosensory cells (hair cells, neurons) and non-neurosensory cells. Hair cells can degenerate because of age, ototoxic drugs, acoustic trauma or genetic predisposition. Two principle approaches have been proposed to restore hair cells that do not regenerate: a cell based therapy and a gene based therapy. For the former, it is essential to differentiate inner ear hair cell progenitors before exploring their replacement capability when transplanted in in vitro/in vivo models of hearing loss.

Our primary goal is to develop an efficient and reliable protocol that recapitulates in vitro the developmental steps involved in the generation of the ear primordium, the otocyst, and induces the differentiation of mouse embryonic stem cells (mESCs) into hair cell progenitor cells.

During normal development, hair cell differentiation requires Math1, the mammalian atonal homologue (Bermingham, et al. Science, 284, 1999). For our study, we used a mESC line derived from the Math1-GFP reporter mouse. The Math1-GFP transgene is expressed exclusively in the hair cells, which qualifies these transgenic mice as an important tool to easily characterize the potential of hair cell progenitors generated from these reporter mESCs based on their ability to give rise to GFP-expressing hair cell progenitors. Our in vitro differentiation protocol is based on generating ectoderm

from mESC-Math1 GFP cells, by inhibiting the formation of the primitive streak, a structure that is essential for the induction of the endoderm and the mesoderm during mammalian gastrulation. More specifically, Wnt and TGF- β signaling pathways suppress primitive streak and increased presumptive ectoderm. Embryoid bodies, generated from mESC-Math1 are treated with Wnt inhibitor (Dkk1), Smad3 inhibitor (SIS3) and IGF1. The generated ectodermal cells are then exposed to known otic inducers (i.e. FGF) and tested for the expression of hair cell progenitor markers. The expression of selected known markers (i.e., Pax2, Pou4f3, Myosin VIIa), for otic cell fate are quantified by immunostaining and Q-PCR analyzes. In parallel, we performed a pilot study exploring the potential of Math1-GFP cells, isolated from differentiated embryoid bodies, to differentiate into hair cell like-cells, after transplantation into cochlear organ explants.

Poster Board Number: I26

INVESTIGATING THE ROLE OF INFLAMMATORY SIGNALING AND RUNX1 IN PROSTATE HOMEOSTASIS, RESPONSE TO INJURY AND CANCER INITIATION

Liu, Xian¹, Soroudi, Camille¹, Stoyanova, Tanya¹, Witte, Owen¹, Mikkola, Hanna², Goldstein, Andrew¹

¹Molecular and Medical Pharmacology, UCLA, Los Angeles, CA, USA, ²Molecular, Cell and Developmental Biology, UCLA, Los Angeles, CA, USA

Inflammation and tissue injury in the prostate induces a proliferative response in a subset of epithelial cells, likely enhancing their susceptibility to prostate cancer. While much evidence points to progenitor cells within the basal layer, we find that inflammation is associated with the acquisition of a luminal progenitor population with reduced androgen signaling and increased clonogenic capacity. In the human prostate, we have identified an antigenic profile to routinely isolate inflammation-associated luminal progenitor cells from patient tissue. To model this process in the mouse, we have utilized surgical castration which promotes inflammation into the prostate and a reduction in epithelial androgen signaling. Transcriptional profiling identified elevated expression of the self-renewal regulator Runx1 in both human prostate luminal progenitor cells and in the castrated mouse prostate. Over-expression and knockout studies demonstrate that Runx1 regulates normal mouse prostate progenitor self-renewal and proliferation in vitro. In vivo studies are underway to determine the role of inflammation in the transformation of luminal progenitor cells, and the role of Runx1 in tissue injury and repair following inflammation. We are further studying the mechanisms by which Runx1 regulates prostate progenitor function. Evaluation of a range of mouse and human prostate cancer models identified elevated Runx1 expression in advanced and aggressive prostate cancer, suggesting a role for this factor in both the normal and malignant prostate.

Poster Board Number: I27

THE ROLE OF THE DE NOVO DNA METHYLTRANSFERASES DURING EPIDERMAL STEM CELLS DIFFERENTIATION

Rinaldi, Lorenzo, Solanas, Guiomar, Morey, Lluís, Di Croce, Luciano, Aznar-Benitah, Salvador
CRG, Barcelona, Spain

DNA methylation is an epigenetic mechanism essential for mammalian embryonic development and adult tissue homeostasis. In mammals, DNA methylation is catalyzed by the three DNA methyltransferases DNMT1, DNMT3A and DNMT3B. DNMT3A and DNMT3B are de novo DNA methyltransferases establishing DNA methylation profiles during embryogenesis, but they also have a role in maintaining DNA methylation together with DNMT1. Recent findings indicate that the effect of DNA methylation on gene expression is variable, and while promoter methylation is associated with gene repression, gene body methylation relates to transcriptional elongation.

One of the interests of our lab is to understand the epigenetic

mechanisms that regulate epidermal stem cell (EpSCs) function. We have obtained the global changes in the transcriptome of human epidermal stem cells during their stepwise differentiation. These results revealed that Dnmt3a and Dnmt3b show opposite patterns of expression during the process of epidermal stem cell differentiation. While the expression of Dnmt3a was upregulated, Dnmt3b expression decreases as epidermal stem cells commit to differentiation. Knock-down of the two methyltransferases significantly perturbed the self-renewal of human epidermal stem cells, and the increased expression of differentiation markers as shown by global transcriptome analysis through RNA-sequencing. Interestingly, Dnmt3a and Dnmt3b might also be important for restricting lineage determination as mesodermal genes, that are normally silenced in keratinocytes, become expressed de novo in Dnmt3a or Dnmt3b KD cells. By ChiP-Seq analysis we have found that both proteins show little overlap in their genomic occupancy, and selectively bind to a large number of protein coding and non-coding RNAs genes, either at the promoter or within the gene body. The binding to these is highly dynamic and dramatically changes between stem cells and their differentiated counterparts. Interestingly, DNMT3A and DNMT3B are expressed in different compartments in murine skin with little overlap. We have generated epidermal conditional knockout mice of both proteins which indicates that deletion of each DNA methylase results in different skin phenotypes. Altogether our results highlight the selective function both de novo DNA methylases in vivo, and their importance in mammalian epidermal homeostasis.

Poster Board Number: I28

MODELING INNER EAR ORGANOGENESIS USING MOUSE EMBRYONIC STEM CELLS

Koehler, Karl R.¹, Mikosz, Andrew M.¹, Molosh, Andrei I.², Patel, Dharmeshkumar¹, Hashino, Eri³

¹Stark Neurosciences Research Institute, Indiana University School of Medicine, Indianapolis, IN, USA, ²Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN, USA, ³Department of Otolaryngology-Head and Neck Surgery, Indiana University School of Medicine, Indianapolis, IN, USA

Sensory epithelia in the inner ear contain specialized mechanosensitive hair cells that detect auditory and vestibular stimuli. Previous attempts by other investigators to derive sensory hair cells in the inner ear have been unsuccessful due to inefficient and incomplete phenotypic conversion of stem cells into inner ear-like cells. A key insight lacking from previous studies is the importance of the non-neural and pre-placodal ectoderm, two critical precursors during inner ear development. We have established a novel approach to generate authentic inner ear sensory epithelia harboring significant numbers of functional sensory hair cells from aggregates of mouse embryonic stem cells (ESCs) in a defined 3D culture. By faithfully recapitulating in vivo inner ear induction with precise temporal control of BMP, TGF β and FGF signaling, ESC aggregates sequentially give rise to non-neural, pre-placodal and otic placode-like epithelia. Adjacent to these ectodermal epithelia we consistently found formation of mesenchymal tissues, mimicking the in vivo periotic microenvironment. Remarkably, in a self-organized process, vesicles containing inner ear prosensory cells subsequently emerge from the presumptive otic placodes and give rise to hair cells bearing stereocilia bundles and a kinocilium. These stem cell-derived hair cells exhibit functional properties of native mechanosensitive hair cells and form specialized synapses with neurons that have also arisen from ESCs in the culture. We anticipate that this novel in vitro model can be used to gain deeper insight into inner ear development and disorder. Moreover, this model could be modified to study formation of other complex organs requiring mesenchymal-epithelial interactions.

Poster Board Number: I29

CORNEAL RECONSTRUCTION WITH TISSUE-ENGINEERED HUMAN ORAL MUCOSAL EPITHELIAL CELL SHEETS USING AUTOLOGOUS MESENCHYMAL CELL FEEDER LAYERS AND AUTOLOGOUS SERUM

Oie, Yoshinori¹, Soma, Takeshi¹, Maeda, Kazuhisa², Hayashida, Yasutaka¹, Hayashi, Ryuhei¹, Sugiyama, Hiroaki¹, Shirakabe, Masayuki³, Kikuchi, Akihiko⁴, Yamato, Masayuki⁴, Sawa, Yoshiki³, Okano, Teruo⁴, Nishida, Kohji¹

¹Department of Ophthalmology, Osaka University Graduate School of Medicine, Suita, Japan, ²Department of Complementary and Alternative Medicine, Osaka University Graduate School of Medicine, Suita, Japan, ³Medical Center for Translational Research, Osaka University, Suita, Japan, ⁴Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan

Purpose: Tissue-engineered cell sheets composed of autologous oral mucosal epithelium have been successfully used to reconstruct eyes affected with total corneal stem-cell deficiency. However, the use of murine feeder cells and fetal bovine serum during culture process has the potential risk of transmitting animal infectious diseases. In the current study, we report corneal reconstruction with tissue-engineered oral mucosal epithelial cell sheets using autologous mesenchymal cell feeder layers and autologous serum cultured in Good Manufacturing Practice grade.

Methods: Six patients with total limbal stem-cell deficiency were enrolled in this study. Five of the patients were female, and one was male. Oral mucosal epithelial cells were cultured with autologous X-ray-treated adipose-derived mesenchymal cells, and autologous serum on temperature-responsive culture inserts in Good Manufacturing Practice conditions. Oral mucosal epithelial cell sheets were harvested with temperature reduction, and transplanted to patient's corneal surface. The primary endpoint was the epithelization and transparency grading of the central cornea in 7 mm diameter. The secondary endpoint was best corrected visual acuity.

Results: Postoperative epithelization and transparency grading at one year (0.33 ± 0.52) was significantly better than preoperative grading (3.0 ± 0.0) ($P=0.031$, Wilcoxon Signed rank test). Visual acuity at one year was significantly better than preoperative visual acuity ($P=0.005$, paired t-test). No severe adverse events were reported. **Conclusions:** The tissue-engineered oral mucosal epithelial cell sheets using autologous mesenchymal stem cell feeder layers and autologous serum cultured in Good Manufacturing Practice grade are effective and safe to reconstruct the corneal epithelium in patients with total limbal stem-cell deficiency.

Poster Board Number: I30

TOWARDS STEM CELL BASED THERAPY FOR DISEASES WHICH AFFECT THE RETINAL PIGMENT EPITHELIUM

Bennis, Anna¹, Gorgels, Theo G.M.F.¹, Heine, Vivi M.², Bergen, Arthur A.B.¹

¹Molecular Ophthalmogenetics, Netherlands Institute for Neuroscience, Amsterdam, Netherlands, ²Stem Cell Laboratory, VU University Medical Center, Amsterdam, Netherlands

The neural retinal pigment epithelium (RPE) is a monolayer of pigmented cells forming a part of the blood/retina barrier. The RPE closely interacts with the photoreceptors in the maintenance of visual function. Pathological involvement of the RPE in retinal diseases such as age related macular degeneration (AMD), Stargardt disease or retinitis pigmentosa, shows the importance of these cells. A possible new therapy for AMD might be developed by tissue engineering, using in vitro differentiating stem cells in RPE-like cells which can be used for transplantation. A number of groups were previously able to produce stem cell derived RPE-like cells and transplant these in an animal model of AMD leading to improvement in visual performance. However the differentiation protocols used at this time for in vitro differentiation towards RPE-like cells have a low efficiency and rely for a large part on spontaneous differentiation.

In order to increase the efficiency and specificity of the protocol for differentiation of stem cells in to RPE cells, we gathered and applied data of in vivo gene expression in the retina to monitor the stem-

cell-to-RPE-cell differentiation. We used a new strategy to define and characterize the transcriptome of the RPE: we determined the transcriptomes of the RPE, photoreceptors and choroid. By subtracting possible contaminating RNA of the adjacent layers, we obtained a purified list of genes specifically expressed in the RPE. We did this for mouse and human RPE. The RPE signature genes list is being used to monitor the RPE development in vitro and in vivo.

Poster Board Number: I31

EFFECTIVE TRANSPLANTATION OF PHOTORECEPTORS DERIVED FROM THREE-DIMENSIONAL CULTURES OF EMBRYONIC STEM CELLS

Gonzalez-Cordero, Anai¹, West, Emma L.¹, Pearson, Rachael A.¹, Duran, Yanai¹, Carvalho, Livia S.¹, Chu, Colin J.¹, Naeem, Arifa¹, Blackford, Samuel J.¹, Georgiadis, Anastasios¹, Lakowski, Jorn², Hubank, Mike³, Smith, Alexander J.¹, Bainbridge, James W B¹, Jane C Sowden², Robin R Ali¹

¹Genetics, UCL Institute of Ophthalmology, London, United Kingdom, ²UCL Institute of Child Health, London, United Kingdom, ³Genomics, UCL Institute of Child Health, London, United Kingdom

Irreversible blindness caused by loss of photoreceptors may be amenable to cell therapy. We have previously demonstrated retinal repair¹ and restoration of vision through transplantation of photoreceptor precursors obtained from postnatal retinas into visually impaired adult mice^{2,3}. Considerable progress has been made in differentiating embryonic stem cells (ESCs) in vitro toward photoreceptor lineages⁴⁻⁶. However, the capability of ESC-derived photoreceptors to integrate after transplantation has not been demonstrated unequivocally. In order to isolate photoreceptor precursors fit for transplantation, we have adapted a recently reported three-dimensional (3D) differentiation protocol that generates neuroretina from mouse ESCs⁶. Here, we show that a pure Rhodopsin.GFP population of rod precursors can integrate within degenerate retinas of adult mice and mature into outer segment-bearing photoreceptors. Notably, ESC-derived precursors at a developmental stage similar to postnatal days 4-8 integrate more efficiently compared with photoreceptors at more mature stages. This study shows conclusively that ESCs can provide a source of photoreceptors for retinal cell transplantation.

Poster Board Number: I32

CONDITIONED MEDIA FROM IPS CELLS ATTENUATE BLEOMYCIN INDUCED LUNG INJURY IN THE RAT LUNG BY HGF

Gazdhar, Amiq¹, Grad, Iwona², Gugger, Mathias³, Feki, Anis⁴, Geiser, Thomas¹

¹Department of Pulmonary Medicine, University Hospital Bern, Bern, Switzerland,

²Department of Gynecology, University Hospital Geneva, Geneva, Switzerland,

³Department of Pathology, University of Bern, Bern, Switzerland, ⁴Department

Abstetrics and Gynecology, Freiburg Hospital, Freiburg, Switzerland

Background: Idiopathic pulmonary fibrosis (IPF) is a progressive and irreversible fibrotic process and a major cause of death. The progressive fibrosis is a result of complex series of interdependent events that occur in response to repeated alveolar epithelial injury. Recently, cell based therapies in regenerative context have been investigated as a novel therapeutic approach, however with limited success. Induced pluripotent stem cells (iPSCs) and their secreted mediators offer a novel and promising potential treatment due to their regenerative properties. As a first proof of concept we evaluated the potential of human iPSCs conditioned media (iPSCs-cm) to regenerate the alveolar epithelium in bleomycin injured rat lungs.

Methods: iPSCs-cm was collected from the culture of iPSCs derived from human foreskin fibroblasts and its effect on alveolar epithelial wound repair was tested in in vitro wound healing assay, furthermore iPSCs-cm was instilled intra tracheally into bleomycin injured rat lungs to study its effect in vivo.

Results: High levels of hepatocyte growth factor (HGF) were detected

in iPSCs-cm. iPSCs-cm increased alveolar epithelial wound healing in vitro compared to control. Moreover, intratracheal instillation of iPSCs-cm further reduced the collagen content and improved histology. Furthermore, TGF β 1 levels and α -smooth muscle actin (sma) expression were markedly reduced in the iPSCs-cm compared to control groups.

Conclusion: Compared to control iPSCs-cm increases alveolar epithelial wound repair in vitro and attenuates bleomycin induced fibrosis in vivo, partially due to presence of HGF and may represent a promising novel cell free therapeutic option for lung injury and fibrosis.

Poster Board Number: I33

HUMAN AND MURINE ORAL MUCOSA STEM CELLS EXHIBIT SIMILAR PHENOTYPES - LESSONS FOR TRANSLATIONAL STUDIES

Arie, Ina, Kuperman, Shiri, Treves, Sandra, Buch, Sigal, Pitaru, Sandu
Oral Biology, School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel

An important step in translating a newly conceived drug (chemical or biological in nature) from the bench to the clinic is to test its safety and efficacy in animal models. Because of their pleiotropic effects, stem cells (SC) and their derivatives can be considered complex biological drugs. Usually, their translation requires testing in an animal model. Due to their low cost and particularly to the availability of numerous transgenic phenotypes, murine models are extensively used in translational medicine.

Oral mucosa is a readily accessible source for SC. We have recently identified a primitive neural crest-like SC population in the lamina propria of the adult human oral mucosa termed hOMSC. hOMSC are highly expandable with no change in their phenotype, express constitutively substantial levels of the pluripotency associated markers Oct4, Sox2 and Nanog, the neural crest markers snail and p75 and the neural SC markers Sox2, nestin and Pax6. hOMSC differentiate in vitro into cell lineages of the three germ layers and develop into the majority of neural crest derived tissues in nude mice. hOMSC-derived dopamine secreting cells ameliorate Parkinson's symptoms in cyclosporine-treated hemi-Parkinsonian rats. These hOMSC properties open a wide spectrum of therapeutic interventions, in which harvesting complexities and the ethical and safety implications associated with either embryonic SC, iPS or fetal SC can be avoided. However, their translation requires the use of immunodeficient animal models or animal treatment with immunosuppressive drugs that might affect the results and the interpretation of therapeutic protocols.

Thus, the purpose of the present study was to test whether murine-derived oral mucosa SC – mOMSC express a phenotype similar to that of hOMSC.

hOMSC and mOMSC were derived from the lamina propria of the oral palate after obtaining approval of Institutional Helsinki and Animal Ethics Committees and expanded in DMEM (low glucose) + 10% FCS. Both cell types were tested for their growth potential, expression of SC markers and the capacity to differentiate in vitro into cell lineages derived from the 3 germ layers.

It was found that the doubling time of mOMSc is by 2 folds higher than that of hOMSC (96 vs. 50 hr). The cloning efficiency was ~ 60% for both cell types. Flow cytometry indicated that both cell types were similarly positive for Nanog (~35-40%), Sox2 (65-70%) and nestin (~70%). However, the number of Oct4⁺ cell was reduced in mOMSC cultures (30% mOMSC vs. 70% hOMSC). Immunofluorescence and immunohistochemistry indicated that the vast majority of both cell types were positive for alkaline phosphatase and nestin. These results were confirmed at the molecular level by RT-PCR.

Osteogenic medium (dexamethasone, β -glycerolphosphate, vit C) induced the formation of mineralized nodules in cultures of both cell types, even though this process was faster in mOMSC (3 vs. 6 weeks). Activin A induced the development of definitive endoderm in both cell types as assessed by the expression of Sox17 and Foxa2 at the protein and molecular levels. dbcAMP, IBMX, Neuregulin and PDGF induced the formation of astrocytic-like cells.

The results of this study indicate that albeit minor changes the phenotype of mOMSC is similar to that of hOMSC. Thus, it is suggested that mOMSC can replace hOMSC for initial translational studies aimed at testing the therapeutic effect of oral mucosa derived SC.

Poster Board Number: I34

ROLE OF NOTCHLESS IN SKELETAL MYOGENESIS

Gayraud-Morel, Barbara¹, Le Bouteiller, Marie², Stedman, Aline², Cohen-Tannoudji, Michel², Tajbakhsh, Shahragim¹

¹*Stem Cells and Development, Institut Pasteur, Paris, France*, ²*Génétique Fonctionnelle de la souris, Institut Pasteur, Paris, France*

Ribosomes have been reported to play novel roles in the differential regulation of cell behaviour; consequently, they are no longer considered as passive members of the constitutive translational machinery, but rather as complexes that provide an additional level of regulation of gene expression. Notchless (Nle) is a highly conserved nuclear WD40 repeat protein. It was named from a phenotype related to the Notch pathway in *Drosophila*, but it was recently suggested that its function is associated with ribosome biogenesis. Recent studies using tissue specific deletion of Nle have suggested that this protein is required for the maintenance of adult hematopoietic stem cells (Le Bouteiller et al., 2013). The importance of specific ribosomal proteins is further demonstrated in DBA (Diamond-Blackfan anemia) patients, where haploinsufficiency of ribosomal protein results in severe anemia. In this study, we analyse Nle function in satellite (stem) cells and myoblasts. Using conditional mutant mice, Nle^{fllox}/lacZ, we induced in vitro and in vivo Nle deletion by expressing a ubiquitous or a muscle stem cells specific CreERT2, generating R26CreERT2:Nle^{fllox}/lacZ mice and Tg-Pax7CreERT2::Nle^{fllox}/lacZ respectively. We show that addition of hydroxy-tamoxifen in vitro on freshly isolated satellite cells, induces rapidly Nle deletion and an arrest in proliferation. However, deletion of Nle in differentiated myoblasts or myotubes does not impair myogenesis. Tamoxifen injections in mice induced specific deletion of Nle in satellite cells. In absence of Nle, satellite cell quiescence was not affected, at least for a period of 3 months, but over longer periods satellite cell function was compromised. Culture of in vivo deleted cells showed that the majority of cells were not proliferating due to major defects in ribosome formation.

We are currently investigating if all satellite cells respond equally to the loss of Nle function by an absence of proliferation, and if this phenotype is mediated by the p53 stress response pathway.

Poster Board Number: I35

TRANSLATIONAL PROCESS OF THE PALL CELERIS PRODUCT FOR CRITICAL LIMB ISCHEMIA

Spaltro, Gabriella¹, Gambini, Elisa¹, Bassetti, Beatrice¹, Quarti, Cinzia², Capogrossi, Maurizio C.³, Pompilio, Giulio¹, Pompilio, Giulio⁴

¹*Vascular Pathology and regenerative Medicine Laboratory, Centro Cardiologico Monzino, Milan, Italy*, ²*Pall Italia, Buccinasco (Milan), Italy*, ³*Istituto Dermatologico dell'Immacolata, Rome, Italy*, ⁴*Dipartimento di scienze Cliniche e di Comunità, Università di Milano, Milan, Italy*

Background: Critical Limb ischemia (CLI) is the end-stage of peripheral arterial disease (PAD). Clinical symptoms include pain at rest, ulceration and necrosis. CLI is also associated with a very high mortality rate (25% at 1 year), even greater after amputation (40%). We are considering treating CLI patients with multidosing intramuscular autologous injections of total nucleated cells (TNCs), obtained after peripheral whole blood gravity filtration using Pall Celeris™ System. Aim of the project is to characterize these cells in order to describe their mechanism of action and to prove they can be used as a transplant. In fact, according to an ISS algorithm, a cellular product is regulated as transplant if: contains intact cells, cells are not genetically or physiologically modified and are used in a homologous way. We aim to demonstrate that these cells are able to migrate, not trans-differentiate into endothelial cells and exert their biological

regeneration with a paracrine indirect effect.

Methods: TNCs were isolated from non-pathologic whole blood using Pall Celeris™ gravity filtration system. Anti-human CD3, CD19, CD14, CD66b, CD34 and KDR were used to test by flow cytometry the enrichment of specific blood population after filtration. Recovery was calculated as ratio of the total TNCs before and after filtration. To evaluate the migration ability 5×10^5 TNCs were plated in the upper chamber of a 24w transwell. In the lower chamber was seeded M199 medium conditioned with VEGF (50ng/ml) or SDF-1 (100ng/ml) and after 24 hours it has been evaluated the number of cells in the lower face of the filter and in the lower chamber of the transwell. To test capillary tube formation 1×10^5 TNCs were seeded in EGM-2 medium onto Cultrex coated plates and cells were monitored daily, up to 7 days for capillary-like structure formation. Endothelial differentiation was evaluated seeding 3×10^5 TNCs in M199, 10% FBS onto fibronectin coated plates and confirming after 7 days the presence of KDR, vonWillebrand Factor and VeCadherin antigen. Serum from whole blood samples and supernatant medium after culture were collected to assess proangiogenic factors release using Luminex technology. Results: TNCs mean recovery using Pall Celeris™ System is $57,57 \pm 22,35$. Flow cytometry characterizations show an enrichment of each population after filtration (CD3 $7,78 \pm 5,12$, CD14 $5,25 \pm 3,21$, CD19 $4,75 \pm 5,69$, CD66b $4,72 \pm 0,22$, CD34 $5,65 \pm 4,18$ and KDR $18,07 \pm 19,92$ respectively). Migration assay shows that TNCs migrate towards a VEGF or SDF-1 gradient ($1,847 \pm 0,637$ and $1,983 \pm 0,856$ ratio of migrated cells vs negative control respectively). Capillary tube formation assays were negative and endothelial differentiation assays showed no differences after filtration. The release testing of angiogenic paracrine factors is still in progress.

Conclusions: Pall Celeris™ System is a simple, effective, and point-of-care system to obtain TNCs from peripheral blood. Basing on these preliminary results, we showed that the cells obtained with Pall Celeris™ System may be considered as a transplant and suitable for being tested as angiogenic effectors in an ischemic context.

Poster Board Number: I36

THE EFFECT OF VEGF AND SERUM-DEPRIVATION ON PHENOTYPE AND FUNCTION OF ADIPOSE-DERIVED STROMAL CELLS FROM PATIENTS WITH CHRONIC ISCHEMIC HEART DISEASE COMPARED WITH HEALTHY DONORS

Tratwal, Josefine¹, Follin Larsen, Bjarke¹, Haack-Sørensen, Mandana¹, Elberg, Jens Jørgen², Kastrup, Jens¹, Ekblond, Annette¹

¹Cardiology Stem Cell Center, The Heart Center, Rigshospitalet, University Hospital Copenhagen, Copenhagen, Denmark, ²Department of Plastic Surgery, Rigshospitalet, University Hospital Copenhagen, Copenhagen, Denmark

Purpose: A novel approach for treating patients suffering from chronic ischemic heart disease (IHD) who do not respond to conventional care is stem cell therapy with potential to support regeneration of the damaged myocardium. Adipose-derived stromal cells (ASCs) stimulated with vascular endothelial growth factor (VEGF) are currently applied in the first in-man, double-blind, placebo-controlled MyStromalCell Trial as treatment therapy for IHD patients. The present in vitro study investigates the effect of VEGF and serum deprivation on ASCs from IHD patients (n=7) compared with those from healthy donors (n=7), in terms of differentiation toward endothelial lineage. Methods: ASCs were stimulated with 50µg/ml VEGF in serum-deprived medium containing 2% fetal bovine serum (FBS) for one to three weeks, and compared with ASCs cultured in complete medium (10% FBS) or serum-deprived medium (2% FBS). The expression of stem cell markers, endothelial markers and VEGF-receptor 1 was measured with real-time quantitative PCR, flow cytometry and visualized by immunocytochemistry. Additionally, functional tube formation and proliferation rates were assessed.

Results: Serum-deprived and VEGF-stimulated ASCs had significantly increased transcriptional expression (p<0.05) of early endothelial marker Forkhead box F1, endothelial marker von Willebrand Factor,

and VEGF-receptor 1 compared with ASCs from complete medium. No changes were observed on protein level, however tube formation occurred on ECMatrix® for ASCs in VEGF-stimulation and serum-deprived medium. The only observed difference between IHD patient and healthy donor ASCs was a slightly decreased proliferation rate for the latter. ASCs maintained their stem cell characteristic markers (p<0.05) under all conditions.

Conclusions: Serum-deprived ASCs from IHD patients and healthy donors were equally predisposed toward endothelial lineage while maintaining their stem cell characteristics, with no additional effect of VEGF stimulation.

Poster Board Number: I37

AN ACE2 ACTIVATOR IMPROVES PROGENITOR CELL FUNCTIONS IN A RAT MODEL OF HEART FAILURE

Qi, Yanfei¹, Cole-Jeffrey, Colleen², Zhang, Juan¹, Shenoy, Vinayak¹, Espejo, Andrew¹, Song, Chunjuan¹, Hanna, Mina³, Pepine, Carl⁴, Katovich, Michael¹, Raizada, Mohan²

¹Pharmacodynamics, University of Florida, Gainesville, FL, USA, ²Physiology and Functional Genomics, University of Florida, Gainesville, FL, USA, ³Materials Science & Engineering, University of Florida, Gainesville, FL, USA, ⁴Medicine, University of Florida, Gainesville, FL, USA

Background: The number and function of cardiac progenitor cells (CPCs) are significantly decreased in patients with ischemic heart disease. Endothelial progenitor cell (EPC) numbers also declines with heart failure (HF) progression and EPCs from patients with chronic ischemic cardiomyopathy are dysfunctional. Treatments for HF with agents such as Angiotensin converting enzyme inhibitors and Angiotensin II receptor blockers suggest an imbalance of renin angiotensin system (RAS) in HF. It has been shown that ACE2 overexpression in the human umbilical vein endothelial cells can lead to an increase in tube formation and promotes endothelial cell migration. ACE2 overexpression has been reported to promote capillary formation and neovessel maturation. We have previously demonstrated that Diminazine acetate (DIZE), a small molecule, increases ACE2 activity. Thus, we hypothesized that activation of endogenous ACE2 by DIZE, would be improve progenitor cell function and attenuate ischemia-induced cardiac pathophysiology.

Methods: DIZE treatment (15 mg/kg/day, s.c.) was initiated in 8-week-old rats two days prior to MI surgery and continued throughout the study-period. Cardiac function was measured four weeks post-MI. The number of circulating rat EPCs (CD4⁺CD5⁺CD8⁺CD90⁺) in the blood was quantified via flow cytometry. Migratory function of EPCs isolated from spleen and bone marrow mononuclear cells was assessed. Engraftment of CPCs in the heart was quantified by immunostaining and western-blot using anti-Islet-1 antibodies (a marker for CPCs).

Results: DIZE caused a two fold increase in the CPCs in the peri-infarct region of the DIZE-treated ischemic heart (Control: 0.37 ± 0.002 ; DIZE: 0.37 ± 0.006 ; MI: 0.21 ± 0.020 ; MI+DIZE: 0.45 ± 0.035 ; p<0.05). This increase in the CPC number suggests a higher regenerative potential in the hearts from DIZE-treated animals. Moreover, the 40% reduction of circulating EPCs observed in the MI group was restored to normal with DIZE treatment (Control: $4.5 \pm 0.2\%$; DIZE: $4.2 \pm 0.5\%$; MI: $2.7 \pm 0.2\%$; MI+DIZE: $4.4 \pm 0.2\%$; % of total MNC; p<0.05). In addition, DIZE improved EPC migratory ability by 40% in the MI rats (Control: 32.3 ± 17.8 ; MI: 20.0 ± 6.5 ; MI+DIZE: 28.6 ± 8.5 ; % increase in migration towards SDF-1α). A significantly reduction (one fold) in capillary density was observed in MI hearts (Control: 68.8 ± 7.6 ; DIZE: 68.8 ± 6.2 ; MI: 33.6 ± 3.2 ; # of capillary vessels per 0.32mm^2 ; p<0.05). DIZE treatment in the MI animals significantly prevented the decrease in the capillary density of the MI group (MI+DIZE: 57.6 ± 3.2). Additionally, DIZE provided impressive protection against MI by preserving fractional shortening, attenuating the increase in left ventricular end diastolic pressure, preventing the dysfunctional changes in contractility, and decreasing infarct size and apoptosis.

Conclusions: Collectively, DIZE causes an increase in CPCs homing

to the peri-infarct area of the ischemic heart, increases circulating EPCs and restores capillary density, all of which are correlated with an improvement of cardiac function. Our results suggest that ACE2 may be critical for progenitor cell function and their ability to attenuate HF.

Poster Board Number: I38

THE CELLULAR DENSITY OF MYELOID ANGIOGENIC CELLS (MACS) DICTATES THEIR ANGIOGENIC FUNCTION

O'Neill, Christina¹, O'Doherty, Michelle¹, Chambers, Sarah E.¹, Guduric-Fuchs, Jasenka¹, Bottazzi, Barbara², Stitt, Alan¹, Medina, Reinhold¹
¹CVS, Queen's University Belfast, Belfast, United Kingdom, ²Laboratorio di Immunofarmacologia, Istituto Clinico Humanitas, Milan, Italy

Purpose: Endothelial progenitor cells (EPCs) have been shown to promote angiogenesis and facilitate vascular repair. Recent clinical trials have demonstrated the feasibility, effectiveness and safety of EPCs for treatment of various ischaemic diseases. However, results from such trials have been inconsistent due to the heterogeneous mix of cells used, as the term EPC has been applied to a broad range of blood derived cells that display endothelial markers and enhance angiogenesis. We have previously characterised two EPC subsets in vitro, myeloid angiogenic cells (MACs) and outgrowth endothelial cells (OECs). Although OECs are the EPC subset with most progenitor characteristics and directly incorporate into the endothelium, MACs have been shown to promote angiogenesis indirectly through paracrine mechanisms. We have recently shown that cell therapy using MACs significantly enhanced vascular repair in a murine model of retinal ischaemia. This MAC response is mediated by the paracrine release of IL8 which transactivates VEGFR2 signalling and induces ERKs phosphorylation (Medina et al. Mol Med 2011;17:1045-55). This pro-angiogenic potential of MACs could be harnessed as a novel cellular therapy for the treatment of ischaemic retinopathy but further optimisation of MACs as a cellular therapy, including optimisation of the density of cells is required.

Methods: A co-culture system of MACs with retinal microvascular endothelial cells (RMECs) in Matrigel was used as an in vitro angiogenesis model to determine MACs effects at various densities. An angiogenesis protein array was then used to identify conditioned media components. The effects of Pentraxin 3 (PTX3) were studied using recombinant PTX3 and an antibody against PTX3.

Results: An in vitro angiogenesis assay demonstrated that MACs significantly increased RMEC tube formation and migratory capacity ($p < 0.01$) when used at a low and mid density. By contrast, there was a significant decrease in RMEC tube formation and migration ($p < 0.01$) when RMECs were co-cultured with a higher density of MACs. Analysis of conditioned media using an angiogenesis protein array demonstrated an up regulation of IL-8 in the low and mid density RMEC-MAC co-cultures. However in the high density MACs group there was a decrease in IL-8 and a concomitant increase in the protein expression of PTX3. In order to determine if PTX3 was responsible for high density MACs anti-angiogenic effects, RMECs were exposed to 100-1000ng/ml of recombinant PTX3. Treatment with PTX3 significantly decreased the capacity of RMECs to form tubules and decreased their migratory capacity ($P < 0.01$). Furthermore, the anti-angiogenic effect of high density MACs was partly abrogated by using an antibody targeted against PTX3 ($p < 0.001$).

Conclusions: We have shown that MACs are capable of inducing angiogenesis in a paracrine manner. However, these data show that at high density, MACs exert an anti-angiogenic effect which is mediated, at least in part, by PTX3. Therefore, density is an important consideration when using cells such as MACs as a therapy for ischaemic disease.

Poster Board Number: I39

THE DERIVATION OF THE CARDIOMYOCYTE AND EPICARDIUM LINEAGES FROM HUMAN PLURIPOTENT STEM CELLS

Witty, Alec¹, Kattman, Steve², Keller, Gordon¹

¹McEwen Centre for Regenerative Medicine, Toronto, ON, Canada, ²Cellular dynamics International, Madison, WI, USA

The epicardium, the outer epithelial layer that surrounds the heart, plays a pivotal role in the development of a fully functional heart during fetal life. Although the origin of this lineage and its role of heart development have been established, the pathways that regulate its development are poorly understood. In this study we have investigated the specification of the human epicardial and cardiomyocyte lineages from pluripotent stem cell differentiation cultures. The epicardial cells specified through cytokine signaling manipulation display morphological characteristics of epithelial cells and express markers associated with the epicardial lineage in vivo, including the transcription factors WT1 and TBX18 and the retinoic acid producing enzyme ALDH1A2. When induced the epicardial cells undergo epithelial-to-mesenchymal transition, giving rise to derivative populations that display characteristics of the fibroblast and vascular smooth muscle lineages respectively. Together, these findings demonstrate the derivation of the epicardial lineage in vitro and in doing so, provide a model for accessing large numbers of human cells for investigating their function in vitro and in pre-clinical models of heart disease in vivo.

Poster Board Number: I40

EX VIVO CHARACTERIZATION AND CARDIOMYOCYTOGENIC POTENTIAL OF ADULT ACTIVATED EPICARDIUM-DERIVED PROGENITOR CELLS

Bollini, Sveva¹, Vieira, Joaquim M.¹, Howard, Sara², Balmer, Gemma², Dubè, Karina N.², Smart, Nicola¹, Riley, Paul R.¹

¹Department of Physiology, Anatomy and Genetics, Sherrington Building, University of Oxford, Oxford, United Kingdom, ²Molecular Medicine Unit, Institute of Child Health, University College London, London, United Kingdom

Background: Ischemic heart disease remains the major causes of morbidity and mortality in the western countries. The epicardium is a layer of mesothelial cells covering the surface of the heart which and during development it actively contributes and regulates the specification of different cardiovascular lineages in the cardiac tissue, including, to some extent, cardiomyocytes. In the adulthood the epicardium becomes quiescent, with loss of proliferation potential and down-regulation of specific embryonic genes, such as WT1. In a previous study we showed that stimulation with the peptide thymosin beta 4 (Tβ4) can reactivate adult epicardium-derived progenitor cells (EPDCs), inducing them to restore their developmental potential with re-expression of embryonic epicardial genes (WT1). The re-activated adult WT1+ EPDCs showed to proliferate and to migrate towards the underlying myocardium, differentiating into new mature and functional cardiomyocytes following ischemic injury.

Aim: The aim of this study is to provide a lineage characterisation of the adult activated EPDCs and their contribution to cardiovascular repair.
Methods: $Wt1^{GFPcre/+}$ mice were primed with Tβ4 solution by intraperitoneal injections and myocardial infarction (MI) was induced by permanent ligation of the left anterior descending coronary artery (LAD). Adult ("activated") EPDCs were obtained from hearts of animals 4 days after MI, via enzymatic digestion and subsequently isolated as $Wt1^{+}GFP^{+}$ cells by FACS-sorting. Embryonic $Wt1^{+}GFP^{+}$ EPDCs were isolated by dissociation of E12.5 hearts of embryos obtained crossing $Wt1^{GFPcre/+}$ male mice with wild type C57Bl/6 females and then FACS-sorted for the expression of GFP. Cells were analysed and characterised by FACS, real time RT-PCR analysis and immunostaining on heart tissue sections.

Results: The adult activated $Wt1^{+}GFP^{+}$ EPDCs showed to be highly heterogenic, expressing cardiac progenitor and mesenchymal stem cell markers, with a phenotypic profile quite dissimilar than their embryonic counterpart. Based on the expression of Sca-1, CD44 and

CD90 antigens, we identified different subpopulations of activated adult WT1+GFP+ EPDCs with distinctive cardiovascular potential and we compared them to the E12.5 WT1+GFP+ EPDCs and the adult GFP- epicardial cells.

In light of these findings, these cells can be considered a novel source of cardiac resident progenitor cells, with clinically relevant potential in regenerative therapy.

Poster Board Number: I41

CO-CULTURES AS TOOLS FOR VASCULARIZATION STRATEGIES

Wolbank, Susanne¹, Holnthoner, Wolfgang², Hohenegger, Karin³, Husa, Anne-Marie³, Mühleder, Severin³, Meinel, Alexandra³, Peterbauer-Scherb, Anja⁴, Redl, Heinz³

¹Ludwig Boltzmann Institute for Traumatology/Trauma Care Consult/Austrian Cluster for Tissue Regeneration, Vienna, Austria, ²Ludwig Boltzmann Institute for Traumatology/ University of Applied Sciences Technikum Wien/ Austrian Cluster for Tissue Regeneration, Vienna, Austria, ³Ludwig Boltzmann Institute for Traumatology/Austrian Cluster for Tissue Regeneration, Vienna, Austria, ⁴Red Cross Blood Transfusion Service of Upper Austria/ Austrian Cluster for Tissue Regeneration, Linz, Austria

In recent years much effort has been made to successfully vascularize engineered tissues. Different approaches like biofabrication of artificial scaffolds, three-dimensional bioprinting or inclusion of different angiogenic growth factors, as well as their time- and space-controlled degradation have been investigated. In addition, co-cultures of different cell types have become interesting candidates for tissue engineering purposes. For in vitro vascularization different cell types were seeded on appropriate scaffolds and analyzed for their ability to form mature, perfusable tube structures. Endothelial cells from different sources (umbilical vein and blood, peripheral blood) in combination with mesenchymal stem cells from e.g. bone marrow or adipose tissue display vascular tube formation in vitro as well as in vivo. Most importantly, these artificially generated microvessels can be perfused and are stably integrated into the host tissue.

Poster Board Number: I42

THE EFFECTS OF SDF1 IN VASCULOGENESIS OF ENDOTHELIAL COLONY FORMING CELLS AND HUMAN FETAL MESENCHYMAL STEM CELLS CO-CULTURE SYSTEM FOR TISSUE ENGINEERED BONE GRAFTS

Teo, Erin Y.¹, Teh, Thomas K.H.², Yang, Joan X.³, Johana, Nuryanti B.¹, Goh, James C.H.², Chan, Jerry K.Y.¹

¹Department of Reproductive Medicine, KK Women's and Children's Hospital, Singapore, Singapore, ²Department of Bioengineering, National University of Singapore, Singapore, Singapore, ³National University of Singapore Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore, Singapore

Of the various strategies devised to enhance vascularization in tissue engineered implants, the inclusion of angiogenic factors, such as vascular endothelial growth factor (VEGF) [1] and basic fibroblast growth factor (bFGF) [2] has been an obvious choice. However, the complex interplay of different key angiogenic factors renders effective transient delivery of multiple factors during vasculogenesis impractical. An alternative approach involves the use of endothelial progenitor cells (EPC). The trafficking and recruitment of circulating EPCs have been understood to be closely mediated by chemokine stromal cell-derived factor-1 (SDF1) expression, as primarily induced and regulated by transcription factor hypoxia-inducible factor-1 (HIF1) in endothelial cells (HUVEC) [3]. Specifically, SDF1 expression is transcriptionally activated through the oxygen dependent stabilization of HIF1 α . With EPCs expressing high levels of CXCR4, they will thus tend to adhere and migrate on hypoxic HUVEC. The SDF1/CXCR4 axis is also the mechanism for homing and migration of mesenchymal stem cells (MSCs) from the bone marrow niche to the repair site [4]. We have thus capitalized the SDF1/CXCR4 axis for vasculogenesis in the

endothelial colony forming cells (ECFC) and human fetal mesenchymal stem cells (hfMSC) co-culture system for tissue engineered bone grafts to treat critical-sized bony defects.

In our pilot study, we have shown that ECFC vessel formation was enhanced in the hypoxic core of the 4mm ECFC-hfMSC constructs in static culture [5]. The effect was however lost in dynamic culture conditions, whereby increased mass transfer occurred across the entire scaffold, indicating the presence of hypoxia induced angiogenic cues secreted by the co-culture system.

In this study, we have thus hypothesized that vasculogenesis is determined by oxygen tension levels acting through HIF1 α activation and secretion of SDF1 in the ECFC-hfMSC co-culture system. The phenotype of ECFC and hfMSC were first characterized in isolation and in co-cultures under hypoxia (2% pO₂ level). Effective SDF1 concentrations were subsequently incorporated to ECFC-hfMSC co-culture in the form of solution or grafted onto microfluidic platform to enable real-time observations of vessel formation across SDF1 gradients. Subsequently, the potential synergistic effects of hypoxia and SDF1 gradients on vasculogenesis in ECFC-hfMSC co-culture were evaluated.

Results showed that HIF1 α and the downstream SDF1 expressions were upregulated in hypoxic hfMSC culture, leading to increased SDF1 secretion. This was coupled with higher proliferation rates and CFU-F ability. The upregulation of SDF1 expression was markedly improved with the introduction of ECFC into the hypoxic co-culture system, leading to the formation of tube-like networks of endothelial structures in vitro. There was also enhanced osteogenic differentiation of hfMSC in the co-culture system, as was supported by our previous work to be credited partially to paracrine signaling of the secreted members in the TGF superfamily. Formation of tube-like endothelial networks was both enhanced and accelerated in the co-culture system with coexisting conditions of hypoxia and SDF1 gradients as compared to either isolated conditions. This demonstrated the synergistic effects of both conditions in generating elevated amounts of SDF1 for vasculogenesis in the ECFC-hfMSC co-culture.

Poster Board Number: I43

A DECELLULARIZED MATRIX AS TISSUE ENGINEERING APPROACH FOR DIAPHRAGM REMODELING OF A MUSCLE-DISEASED MOUSE MODEL

Piccoli, Martina¹, Urbani, Luca¹, Alvarez Fallas, Mario Enrique¹, Dedja, Arben², Franzin, Chiara¹, De Coppi, Paolo³, Pozzobon, Michela¹

¹Fondazione Istituto di Ricerca Pediatrica Città della Speranza, Padova, Italy, ²Dipartimento di Scienze Cardiologiche Toraciche e Vascolari, Università degli Studi di Padova, Padova, Italy, ³UCL Institute of Child Health and Great Ormond Street Hospital, London, United Kingdom

Cellular or gene therapies are often not effective in skeletal muscle degenerative disorders in which impairment of extracellular matrix (ECM) is a major pathological feature, such as muscular dystrophy and other myopathies. In particular, myopathy is especially critical for respiratory (e.g. diaphragm) and limb muscles as they become fibrotic and weak. HSA-Cre, SmnF7/F7 animals present a specific impairment of the skeletal muscle and their diaphragm displays fibrosis and myofiber loss. Since acellular ECM scaffolds retain important bioactive molecules and 3D structure, using a decellularised matrix obtained from healthy-mice diaphragm we aimed at ameliorating diaphragm muscle condition of HSA-Cre, SmnF7/F7 mice. We characterized the decellularised matrix 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 GAG showed a p<0.05 in respect to fresh tissue. Importantly, decellularised matrix possessed the same thickness and stiffness of a fresh diaphragm, and key cytokines such as VEGF and SDF1 α were still present in the acellular muscle. In vivo: decellularised patches were surgically applied to the affected diaphragm of HSA-Cre, SmnF7/F7 mice and changes in terms of

thickness, morphological and cytological aspects were evaluated after 7, 15 and 30 days. 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, with an increasing presence of ki67+ cells. After 30 days the patch was partially reabsorbed while, on the other hand, the weak native diaphragm underwent strong remodeling and increased in thickness.

In conclusion, we successfully developed an acellular diaphragm scaffold and the ECM exerted a positive effect when applied in a myopathic diaphragm influencing local cellular activation, turnover and matrix composition.

Poster Board Number: I44

BONE MARROW-DERIVED MULTIPOTENT STROMAL CELLS AND MONONUCLEAR CELLS TRANSPLANTATION IN RATS WITH POSTINFARCTION CARDIOSCLEROSIS.

Kananykhina, Evgeniya¹, Arutyunyan, Irina², Fatkhudinov, Timur², Bolshakova, Galina¹

¹Laboratory of Growth and Development, Research Institute of Human Morphology of the Russian Academy of Medical Sciences, Moscow, Russian Federation,

²Laboratory of Regenerative Medicine, FSBI "Research Center for Obstetrics, Gynecology and Perinatology" MHS of RF, Moscow, Russian Federation

Objective: Stem cell-based therapy is already widely used for the treatment of cardiovascular diseases. Previous animal and clinical studies have shown the therapeutic benefits of intracoronary injection of bone marrow-derived progenitor cells in acute and chronic heart disease. But the mechanisms of stem cell's efficacy are still unclear. There are a lot of studies confirming the differentiation of transplanted cells to specialized heart cells, but there is also the opposite assumption that only paracrine effect exist.. Our aim was to study engraftment and differentiation of autogenic bone marrow-derived mononuclear cells (MNC) and multipotent stromal cells (BM-MSC) and myocardial reparation after transventricular intracoronary transplantation.

Materials & methods: Acute myocardial infarction (AMI) was induced by transient occlusion (total of 20 min) of the proximal left anterior descending coronary artery and followed by reperfusion. Isolation of MNC and expansion of BM-MSCs were performed by standard protocols. BM-MSCs were CD73, CD90 and CD105 positive and differentiation into three mesoderm-type lineages: osteoblasts, chondroblasts and adipocytes was demonstrated. Cells were labeled with fluorescent tracer PKH26 (Sigma). A total of 30 days after AMI during cross-clamping of aorta the cells were administered into the cavity of the left ventricle at the concentration 5x10⁶/ml in saline solution. In 1 day, 2, 4 and 6 weeks after transplantation the labeled cells were registered in the cryosections and morphometry was performed at the serial paraffin sections of heart.

Results & conclusion: At all stages after injection in both groups transplanted cells were detected only in the scar tissue. The highest level of grafting was observed in MNC group on 14 day after transplantation, but by the 30 day quantity of MNC was decreased whereas MSC's rate remained unchanged. Transplanted cells had a fibroblast-like phenotype and some of them were positively stained for fibroblasts and myofibroblasts specific antibodies: fibroblast activation protein- α and α -smooth muscle actin. The MSC's group had a greater number of stained cells. The infarcted wall thickness was significantly higher in the group with cell transplantation in comparison to control group. But in MNC group this process was accompanied by increase in index of left ventricle dilation indicating the progression of pathological remodeling. The results suggest that engrafted progenitor cells differentiated into myofibroblasts and promoted myocardial fibrosis only in the scar, but not in the perifocal myocardium and they neither differentiated into cardiomyocytes nor into the vascular endothelial cells. Transplantation of MSC is more effective for reverse remodeling of the left ventricle and improvements the myocardial function.

Poster Board Number: I45

SELECTED REGENERATIVE RENAL CELLS ATTENUATE DISEASE PROGRESSION IN RODENT MODELS OF CHRONIC KIDNEY DISEASE THROUGH INHIBITION OF NFKB PATHWAYS AND ENHANCEMENT OF TUBULAR CELL PROLIFERATION

Basu, Joydeep, Bruce, Andrew, Sangha, Namrata, Spencer, Thomas, Guthrie, Kelly, Bertram, Tim, Jain, Deepak
Tengion Inc, Winston-Salem, NC, USA

Regenerative medicine strategies for treating acute and chronic kidney diseases may operate mechanistically by site specific engraftment and directed differentiation of ectopic stem and progenitor cell populations at the site of injury and/or by paracrine signaling networks that mobilize resident cell populations through secretion of regenerative growth factors and micro-vesicles. We have previously described the isolation and characterization of a population of therapeutically bioactive primary renal cells ("selected regenerative renal cells", or SRC), and have demonstrated that SRC can attenuate the progression of chronic kidney disease within rodent models of renal insufficiency. Here, we leverage a combination of in vivo and in vitro functional bioassays to investigate mechanistic pathways for SRC therapeutic bioactivity in the Lewis rat 5/6 nephrectomy model of chronic kidney disease and the ZSF1 model of diabetic nephropathy. We show that direct injection of SRC can reduce chronic infiltration by monocytes/macrophages and T-lymphocytes, typically observed during pathogenesis of fibrotic tissue in both nephropathy models. Moreover, we show SRC can attenuate the NFKB response known to drive tissue inflammation, while simultaneously promoting host tubular cell expansion through trophic cues. Consistent with these in vivo outcomes, we demonstrate that SRC-derived conditioned media can attenuate TNF α -induced NFKB response and increase expression of transcripts associated with cell cycle regulation in the renal epithelial cell line HK2. Observed bioactive responses were independent from extra-cellular vesicles present in SRC conditioned media. Taken together, these data identify a paracrine mechanism by which SRC may provide immuno-modulatory and trophic cues to host renal tissues, thereby catalyzing long-term functional benefits in vivo.

Poster Board Number: I46

STROMAL CELLS OF RENAL CELL CARCINOMA REPRESENT MESENCHYMAL STEM CELL-LIKE CELLS, WHICH PROMOTE EPITHELIAL-MESENCHYMAL-TRANSITION AND TUMOR FORMATION

Börger, Verena¹, Sorg, Ursula R.², Heikau, Sebastian³, Santourlidis, Simeon¹, Ackermann, Rolf³, Wernet, Peter¹, Sorg, Rüdiger V.¹

¹Institute for Transplantation Diagnostics and Cell Therapeutics, Heinrich-Heine-University Hospital, Düsseldorf, Germany, ²Institute for Medical Microbiology and Hospital Hygiene, Heinrich-Heine-University Hospital, Düsseldorf, Germany, ³Institute of Pathology, Vivocell Biosolutions, Heinrich-Heine-University Hospital, Düsseldorf, Germany, ⁴Department of Urology, Heinrich-Heine-University Hospital, Düsseldorf, Germany

Stromal cells are an essential component of tumors. They contribute to tumor development and progression. Moreover, they form a niche for cancer stem cells (CSC) and regulate their activity. Several cell types, including mesenchymal cells, constitute components of tumor stroma. The nature of the mesenchymal cells, mesenchymal stem cells (MSC), tissue fibroblasts or even tumor cells which have undergone epithelial-mesenchymal-transition (EMT), and their role in tumor formation are only poorly understood. Here, we have characterized mesenchymal stroma cells derived from renal cell carcinomas (RCC). The non-permanent GF-1 and DH-1 stromal cell lines have newly been established from metastases of clear cell RCC. They show fibroblast-like morphology, lack cytogenetic aberrations of RCC and do not form tumors when transplanted into immunodeficient mice. In common with MSC and fibroblasts, they express CD10, CD13, CD29, CD44, CD71, CD73, CD90 and CD105 but not CD31, CD34, CD45, CD133, CD326, lin and keratins 8 and 18. Both cell lines can be dif-

differentiated along osteogenic and adipogenic differentiation pathways, an activity they shared with MSC but not fibroblasts. Similarly, their global methylation profile indicates a closer relationship to MSC than to fibroblasts. When co-cultured with epithelial MCF7 mammary carcinoma cells, GF-1 but not or only to a much lesser extent DH-1 cells, MSC and fibroblasts induce EMT. Upon co-transplantation, GF-1 but not DH-1 promotes tumor formation by the normally non-tumorigenic renal cell carcinoma cell line RCC-A in nude, NOD/SCID and NSG mouse models.

Thus, the immunophenotype, differentiation characteristics and global methylation profiles of the RCC-derived GF-1 and DH-1 stromal cells indicate a MSC-like nature of these cells. GF-1 but not DH-1, MSC or fibroblasts induces EMT of MCF7 carcinoma cells and promotes tumor formation by the normally non-tumorigenic RCC-A cell line. These stroma cell lines may help to identify the mechanisms of stromal contribution to tumor development as well as to further define the importance of stroma cells and CSC versus non-CSC in tumor formation.

Poster Board Number: I47

NEW SMALL MOLECULE DRUGS FOR TARGETED CANCER THERAPY

Grzela, Dawid P., Stoczyńska-Fidelus, Ewelina, Lenart, Anna, Pacholczyk, Marcin, Tręda, Cezary, Walczak, Maciej, Winięcka-Klimek, Marta, Piaskowski, Sylwester, Hulas-Bigoszewska, Krystyna, Rieske, Piotr
Celther Polska, Lodz, Poland

Targeted cancer therapies are intended to interfere with cancer cells growth and progression by blocking specific molecular targets involved in functions related to their division, cell movement and response to external signaling. Such therapies are anticipated to be more effective, specific and less harmful for non-cancer cells as compared to classical chemotherapy and radiotherapy.

The targeting approach developed by Celther Polska utilizes chemical compounds interacting with molecular pathways triggered by mutated EGF receptor (EGFRvIII). This mutated growth factor receptor is frequently amplified and overexpressed in prostate cancers and brain tumors.

Until now we have designated wide sets of small molecules and created series of cell lines for in vitro testing. We intend to study the influence of candidate molecules on EGFR pathway by means of high-throughput screening as well as immunocytochemical methods.

Poster Board Number: I48

SPERMATOGONIAL STEM CELLS: A ROUTE TO PRESERVING FERTILITY

Sandher, Raveen K., Pickard, Robert, Herbert, Mary
Newcastle University, Newcastle Upon Tyne, United Kingdom

Over 800 boys are diagnosed with cancer each year and with advances in paediatric oncology survival rates are high [1]. Following chemotherapy treatment sperm production is compromised by depletion of spermatogonial stem cells (SSCs) [2]. Although some recovery may occur sub-fertility frequently persists [3]. Adult males with cancer have the opportunity to preserve fertility by cryopreservation of ejaculated sperm prior to chemo-radiation treatment. However to date pre-pubertal boys with cancer do not have a means of preserving their fertility, as they lack mature sperm. Recent research has raised the exciting possibility that SSCs can be isolated from testicular biopsies, cultured in vitro, and transplanted following cancer therapy to restore spermatogenic capacity [4].

SSC proliferation was slow using established methods for SSC culture. In order to restore fertility large numbers of SSCs will be required to repopulate the testis. I hypothesised that mimicking the in vivo testicular environment by developing a co-culture system with somatic testicular cells may increase proliferation of SSCs. The overall aim was to develop a rapid, reliable and reproducible method for SSC culture

using a mouse model.

SSCs were retrieved from neonatal mouse testes using micro-dissection and cultured on a feeder layer of somatic testicular cells (consisting of sertoli and leydig cells) and gelatin-coated plates. Our findings show that SSCs proliferate rapidly when cultured on a somatic testicular cell feeder layer. This is an important advancement for the future clinical application of SSCs restoring fertility to male childhood cancer survivors.

Poster Board Number: I49

UNDERSTANDING THE ROLE OF THE CANCER STEM CELL-SPECIFIC GENE ASCL1 IN REGULATING GLIOBLASTOMA MULTIFORME INVASIVENESS

Narayanan, Ashwin¹, Mazzoleni, Stefania¹, Gagliardi, Filippo², Cominelli, Manuela³, Bozzuto, Giuseppina⁴, Molinari, Agnese⁴, Poliani, Luigi³, Galli, Rossella¹

¹Neural Stem Cell Biology Unit, Division of Regenerative Medicine, Stem Cells and Gene Therapy, Fondazione Centro San Raffaele, Milan, Italy, ²Neurosurgery, Vita-Salute University, San Raffaele Institute, Milan, Italy, ³Department of Pathology, University of Brescia, Brescia, Italy, ⁴Istituto Superiore di Sanita, Roma, Italy

Glioblastoma multiforme (GBM) is the most malignant and fatal brain tumor of adults. The hallmark of GBM cells is their enhanced invasive ability, which leads to extensive dissemination of tumor cells within the brain. This in turn leads to incomplete surgical removal of the tumor and the persistence of minimal residual disease (MRD). The identification of novel biological mediators specifically regulating GBM invasiveness will provide selective and more efficacious therapeutic approaches to disease treatment.

We performed microarray-based transcriptome profiling of several patient-derived GBM post-surgery specimens and of CSC lines, which were derived from some of these GBM samples and faithfully phenocopy in vivo the tumor of origin and its invasive properties. As controls, we analyzed traditional serum-dependent glioma cell lines (U87, U373, T98G, etc.), which in vivo grow into a large tumor mass, with no discernable invasiveness.

Analysis of the data obtained indicated ASCL1, a proneural transcription factor, to be upregulated in GBM tumors and the corresponding CSC lines. To understand the role of ASCL1 on invasiveness, lentiviral vectors encoding either ASCL1 or the reporter gene GFP as control were used to transduce CSC lines as well as serum grown lines (U87). Interestingly, ASCL1 transduced CSC lines demonstrated a lineage switch in cells, i.e., they shifted from an astrocytic to neuronal lineage both under proliferative and differentiative conditions. Most notably, when the invasiveness was tested using Scanning Electron Microscopy, ASCL1 transduced CSC lines were seen to be more invasive than mock cells and demonstrated collective migration instead of single cell migration. Upon intra cranial transplantation of serum grown and CSC lines, transduced with either ASCL1 or GFP, into nude mice, we observed a decrease in the size of ASCL1 transduced tumors when compared to those generated by mock treated lines. However, we also detected an increase in the dissemination of tumor cells in ASCL1 transduced cell-derived tumors with respect to mock tumors, indicating enhanced invasive ability upon ASCL1 overexpression.

Taken all these results together, we postulate that ASCL1 might act as a potential regulator of invasiveness in GBM.

Poster Board Number: I50

SOX9 FUNCTION IN CANCER

Matheu, Ander, Carrasco, Estefanía, Aldaz, Paula, Garros, Laura, Arrizabalaga, Olatz
Oncology, Biodonostia Institute, San Sebastian, Spain

There is an increasing body of evidences demonstrating that stem cells are implicated in cancer. The misregulation of developmental genes is often associated with cancer, and certain cancers are thought to have a

stem cell origin or to depend on a stem cell (or 'tumour initiating cell') population for their maintenance and/or reoccurrence after therapy. SOX (sex-determining region Y (SRY)-box) genes are transcription factors known to play critical roles during the development of several cell types and tissues in the embryo and to be important for stem cell biology in a number of systems in the adult.

We have demonstrated that SOX9 is overexpressed in a wide range of human cancers. Using both mouse and human cell line studies, and with in vivo mouse tumor models, we observed that SOX9 exhibited several pro-oncogenic properties, including the ability to promote proliferation, inhibit senescence, facilitate migration and invasion and collaborate with other oncogenes in neoplastic transformation. Furthermore, SOX9 overexpression facilitates tumor growth and progression, whilst its inactivation reduced tumorigenicity (1). Mechanistically, we have found that Sox9 directly activates the polycomb Bmi1, whose upregulation represses the tumor suppressor Ink4a/Arf locus. Taken together, these findings provide direct mechanistic evidence of the involvement of SOX9 in neoplastic pathobiology. We will present unpublished data showing a role for Sox9 in cancer stem cells. For this, we characterized SOX9 function in Glioblastoma, the most lethal and common primary brain tumor in adults that contains a population of glioma stem cells (GSCs) essential for tumor origin, maintenance and recurrence.

(1) Matheu, A., et al. (2012). Oncogenicity of the developmental transcription factor Sox9. *Cancer Res* 72, 1301-1315.

Poster Board Number: I51

IDENTIFICATION OF CANCER STEM CELL-LIKE CELLS IN PAPILLARY RENAL CELL CARCINOMA

Börger, Verena¹, Sorg, Ursula R.², Heikaus, Sebastian³, Möhlendick, Birte⁴, Ackermann, Rolf⁵, Wernet, Peter¹, Sorg, Rüdiger V.¹

¹Institute for Transplantation Diagnostics and Cell Therapeutics, Heinrich-Heine-University Hospital, Düsseldorf, Germany, ²Institute for Medical Microbiology and Hospital Hygiene, Heinrich-Heine-University Hospital, Düsseldorf, Germany, ³Institute of Pathology, Heinrich-Heine-University Hospital, Düsseldorf, Germany, ⁴Department of General-, Visceral- and Pediatric Surgery, Heinrich-Heine-University Hospital, Düsseldorf, Germany, ⁵Department of Urology, Heinrich-Heine-University Hospital, Düsseldorf, Germany

Cancer stem cells (CSC) play a crucial role in cancer development. They share unique characteristics with tissue stem cells, such as self renewal, differentiation and expression of stemness genes. Most importantly, however, they are the only cells within a tumor able to initiate and maintain tumor growth. In papillary renal cell carcinoma (pRCC), CSC have not been identified so far.

We have established a tumor cell line, MG-1, from papillary renal cell carcinoma type I. This cell line has cytogenetic aberrations typical for pRCC. It shows epithelial morphology, expresses EpCam and keratins 8 and 18 as well as markers identified on CSC of other tumor entities, e.g. CD29, CD44, CD49f, CD105 and CD133. Moreover, MG-1 cells form serially transplantable tumors in nude, NOD/SCID and NSG mice. Generation of subclones based on CD133 expression reveals that the tumorigenic activity resides within the CD133⁺ cells. Interestingly, CD133⁻ subclones show a mesenchymal morphology and lack EpCam expression whereas CD133⁺ subclones are epithelial in appearance and EpCam⁺. The CD133⁻ subclone MG-1/F7 represents such a CSC-like cell. It has the same cytogenetic aberrations as the parental line, has a unique CD24⁺/CD29⁺/CD44⁺/CD49f⁺/CD105⁺/CD166⁺/c-Met⁺/keratin⁺/EpCam⁺/lin⁻ immuno-pheno-type, shows differentiation along osteogenic but not adipogenic or endothelial pathways, reveals expression of the stemness genes MUSASH1, SOX2 and OCT4, but has no ABC-transporter or aldehyde-dehydrogenase (ALDH) activity. Nevertheless, MG-1/F7 is highly tumorigenic and as few as 1000 cells are sufficient to form serially transplantable tumors. Moreover, MG-1/F7 forms metastases in bone marrow.

Thus, the newly established pRCC cell line MG-1 and particularly its CD133⁻ subclones share typical features with CSC. Importantly,

they indicate that tumorigenicity is the unique characteristic of CSC whereas other markers like CD133 expression or features like ALDH and ABC-transporter activity may be misleading in the identification of CSC of different tumor entities. This cell culture model for pRCC represents a novel useful tool which may contribute to elucidate the molecular pathways involved in the development of pRCC, to develop targeted therapies to CSC and to evaluate the contribution of CSC in tumors to prognosis.

Poster Board Number: I52

INTRAVITREAL ADMINISTRATION OF MULTIPOTENT MESENCHYMAL STROMAL CELLS INCREASES NEUROTROPHIC FACTORS LEVELS AND PREVENTS RETINAL NEURONAL CELL LOSS IN DIABETIC MICE

Ezquer, Marcelo, Simon, Valeska, Conget, Paulette, **Ezquer, Fernando E.**
Instituto de Ciencias, Facultad de Medicina Universidad del Desarrollo, Santiago, Chile

Diabetic retinopathy (DR) is the most common and frightening complication of diabetes mellitus. In developed countries, it is the leading cause of irreversible vision loss. The first symptom of DR is a reduction in color and contrast sensitivity, due to the loss of neuronal cells in the ganglion cell layer of the retina.

Available therapies for DR comprise highly invasive techniques and are applicable only in advanced stages of the disease. Nowadays, no clinical intervention proves to prevent or delay the onset of DR.

Stem cell transplantation appears as a promissory emerging strategy for neuronal degenerative diseases. Multipotent mesenchymal stromal cells (MSCs) are an attractive tool since they could differentiate into neuronal cells and produce neurotrophic factors.

The aim of this work was to evaluate whether the local administration of MSCs lower the loss of retinal neuronal cells in an animal model of DR. For this, mice with type I diabetes mellitus (T1DM mice) received an intravitreal injection of vehicle or 0.2x10⁶ isogenic MSCs at a time when all the pro-damage mechanisms were present but retinal structure still unaffected. Three months later, mice were euthanized and retinal histology (H&E) and gene expression (RT-qPCR) were assessed. As expected, T1DM mice presented a reduced number of nuclei in the retinal layer. However, the number of neuronal cells in the ganglion cell layer of the retina of MSC-treated T1DM mice and normal mice were similar. Concurrently, we observed that the mRNAs levels of bFGF, CTNF, GDNF and NGF genes were increased in the eyes and in the retina of MSC-treated T1DM mice when compared with either untreated T1DM or normal mice.

In order to study the engraftment of MSCs in the retina of T1DM mice, donor cells were isolated from mice that constitutively express GFP in all their tissues (MSC^{GFP}). One, 7, 30, 60 and 90 days after been intravitreally injected, the presence of MSC^{GFP} was assessed (flow cytometry and immunohistofluorescence). At any time point analyzed, donor cells were detected only in the vitreous cavity and they express no neuronal markers.

Thus, we were able to show that MSCs intravitreally administered to T1DM mice survive at least for three months in the vitreous cavity, do not differentiate into neuronal cells but result in an increased production of neurotrophic factors that prevents retinal neuronal cell loss. Therefore, intravitreal administration of MSCs could be envisioned as a therapeutic option to reduce the neuronal cell loss characteristic of the initial stage of DR.

Supported by FONDECYT 1130470 to FE. (eezquer@udd.cl)

Poster Board Number: I53**XENO-FREE ISOLATION AND EXPANSION OF HUMAN AMNIOTIC MESENCHYMAL STEM CELLS**

Gabriel, Christian¹, Lugmayr, Eva¹, Theiß, Denise¹, Witzeneder, Karin¹, Lindenmair, Andrea², Hennerbichler, Simone¹

¹Red Cross Blood Transfusion Service of Upper Austria/ Austrian Cluster for Tissue Regeneration, Linz, Austria, ²Ludwig Boltzmann Institute for Traumatology/ Austrian Cluster for Tissue Regeneration, Linz/Vienna, Austria

Human amniotic membrane represents a rich source of stem cells to be used in regenerative medicine and tissue engineering. Human amniotic mesenchymal stem cells (hAMSC) are isolated by enzymatic digestion using a collagenase solution containing fetal bovine serum (FBS). Isolation is followed by the expansion in media also supplemented with FBS. Besides the animal origin and the batch to batch variations known for FBS, the potential incorporation of xenogenic factors as well as the risk of xeno-immunization in patients makes it unsuitable for clinical use. Due to high cell doses required for application in patients expansion is necessary. Hence protocols according to GMP requirements

Human term placentae were obtained from Caesarean section after informed consent. The amniotic membrane was mechanically separated from chorion and incubated for two hours in a collagenase solution supplemented with or without FBS. The cells were expanded for three passages in media supplemented either with 5% human platelet lysate (hPL) or 10% human serum (huS). The cells were subcultured at a confluence of at least 80% and population doubling times were determined.

hAMSC were isolated from seven human term placentae. Isolation in collagenase solution supplemented with FBS showed no differences in cell counts and morphology compared to the collagenase solution without FBS. In a further attempt the collagenase (for research only) was compared to collagenase suitable for GMP production revealing similar results. Although differences between cells expanded with hPL and huS could be observed, confluence was reached already after three to four days independently of the supplement. Data on population doubling time will be presented.

We were able to establish a GMP-conform protocol for the xeno-free isolation and expansion of hAMSC. Preliminary results indicated that there is no need to add FBS to the collagenase solution for the isolation of hAMSC and that the GMP-grade collagenase is equally suitable. Concerning the expansion, FBS can be substituted by either huS or hPL.

Poster Board Number: I54**HUMAN UMBILICAL CORD DERIVED MESENCHYMAL STEM CELLS AS A NEW SOURCE OF ODONTOBLAST: A PROMISING CELLULAR THERAPEUTIC APPROACH IN REGENERATIVE MEDICINE**

Zanini, Cristina¹, Crudo, Gianluca¹, Mattia, Silvia², Mussano, Federico³, Pizzi, Marco⁴, Tarone, Guido⁵, Forni, Marco¹

¹EuroClone S.p.A Research Laboratory c/o Molecular Biotechnology Centre (MBC), University, Turin, Italy, ²Department of Orthopaedics and Traumatology, University, Turin, Italy, ³Department of Surgical Sciences, Dental School, University, Turin, Italy, ⁴Biomed | Nanotech Development & Business Unit, Eltek Group, Casale Monferrato, Italy, ⁵Department of Molecular Biotechnology and Health Sciences, University, Turin, Italy

Stem cell based regenerative therapy could be considered an innovative approach for curing dental caries. Pulp stem cells from human deciduous teeth (SHEDs) represent a source of committed cells for generating odontoblasts in vitro, anyway SHEDs are not easy to obtain and limited in quantity. Mesenchymal stem cells derived from umbilical cord (UC-MSCs) are considered to be adult stem cells that can be easily obtained in large numbers. The SHEDs and UC-MSCs were conditioned in custom-made serum free culture media in order to induce differentiation toward odontoblasts. SHEDs and UC-MSCs were digested with collagenase and the derived- MSC expanded in vitro. Phenotyped by flow cytometry for specific antigens including

CD29, CD44; CD133, CD105, CD34 and CD45 was performed. SHEDs and UC-MSCs were differentiated in odontoblasts for 21 days with a medium containing FGF, BMP-2 and GDF5. In order to demonstrate odontoblast generation, immunohistochemistry was performed on differentiated cells with antibody against dentin-sialo-phospho- protein (DSPP). Western blotting confirmed the positivity to DSPP in both derived-MSC cells. Morphological immunophenotype and expression of DSPP were comparable in odontoblasts derived from SHEDs and UC-MSCs. The implementation of new scaffolds tailored for dentin regeneration may open new strategies to cure teeth. The capability to induce odontoblast differentiation from unrelated stem cells of unrestricted availability may be a first step toward a new innovative approach including dentin regeneration.

Poster Board Number: I55**CONTROL OF RABBIT MESENCHYMAL STEM CELLS DIFFERENTIATION LINEAGE THROUGH REHABILITATIVE MECHANICAL CONDITIONING REGIME FOR THE DEVELOPMENT OF TISSUE ENGINEERED TENDON/LIGAMENT**

Teh, Thomas K.H.¹, Toh, Siew Lok², Goh, James C.H.³

¹Department of Bioengineering, National University of Singapore, Singapore, Singapore, ²Departments of Bioengineering and Mechanical Engineering, National University of Singapore, Singapore, Singapore, ³Departments of Bioengineering and Orthopaedic Surgery, National University of Singapore, Singapore, Singapore

Physical and mechanical cues have been reportedly shown to control stem cell self-renewal and lineage specification. Particularly, matrix/ substrate stiffness, surface topography, cell shape and mechanical forces have been shown to affect cellular sensitivity and response to the biophysical cues. Mesenchymal stem cells (MSCs) have especially been found to exhibit an acute sensitivity and response to these macroscopic and microscopic cues, indicating the significance of biophysical cues in cell fate control. In providing mechanical cues for tenogenic differentiation of MSCs, the common approach has been to provide leveled physiological loading to condition cell-seeded constructs. This may be sub-optimal as the stimulation intensity may be irrelevant or unsuitable throughout the different development phases. It is thus hypothesized that transient increment of mechanical cues will direct and accelerate tenogenic differentiation of MSCs seeded constructs to realize functional tissue engineered tendons/ligaments in vitro.

A comparative study is performed between three conditioning regimes of continuous levels (high and low intensity) and rehabilitative regime, with static culture as control. Scaffolds of knitted silk fibroin with aligned electrospun silk fibroin were seeded with rabbit bone marrow stromal cells (rBMSCs, P2, 1.5x10⁶/scaffold) and cultured statically for 3 days before loading into a customized bioreactor. Continuous low and high intensity, with different cyclic frequencies, were applied for the two groups ("Continuous low" at 0.1 Hz and "Continuous high" at 0.5 Hz) through the 28 days study. The group undergoing rehabilitative regime ("Rehab") would be exposed to "Low" (Days 3-7, 0.1 Hz) and "High" (Days 7-28, 0.5 Hz) stimulation intensities. All other parameters were constant (5% strain and 12 hrs/day periodic frequency). Results showed that constructs from the "rehab" group had significantly more viable cells when compared to the "continuous high" group and "static" control on day 28 respectively. By day 28, all targeted tendon/ligament-related genes were significantly expressed in the "rehab" group compared to the other groups. This was especially so for tenascin-C and tenomodulin, which were consistently up-regulated in the "rehab" group to trigger collagen fiber build-up and thickening, leading to overall tissue maturation. This was in line with collagen quantification results and histological findings, whereby extensive collagen fiber formation and tendon/ligament-related protein deposition was demonstrated in the "rehab" group by day 28, with the formation of tendon/ligament-like microstructural "crimp" patterns. Through this comparative study, a preliminary outlook of a suitable mechanical stimulation regime was presented. Compared to other regimes, the

"Rehab" regime stimulated proliferation in the earlier stages of the stimulation period, which transcended towards the tenogenic differentiative phase in an accelerated manner after 14 days. Differentiation was marked by the increased collagen deposition, up-regulation of tendon/ligament-related genes and deposition of the corresponding ECM components. The rehabilitative stimulation regime thus provided dynamic cues that were more effective in triggering the formation of collagen fibers and continuously straining these fibers efficiently to achieve collagen fiber thickening and consequently tissue maturation.

Poster Board Number: I56

MYELOMA AND BONE MARROW MESENCHYMAL STEM CELLS' CROSSTALK: EFFECT ON TRANSLATION INITIATION

Attar-Schneider, Oshrat¹, Liat, Drucker¹, Zismanov, Victoria¹, Dabbah, Mahmoud¹, Tartakover-Matalon, Shelly¹, Lishner, Michael²

¹Oncogenetic Laboratory, Meir Medical Center, Kfar-Saba, Israel, ²Internal Medicine and Oncogenetic Laboratory, Meir Medical Center, Kfar-Saba, Israel

Background: The interaction of myeloma (MM) cells with the bone marrow (BM) microenvironment is detrimental to effective treatment. Mesenchymal stem cells (MSC) are an important component of BM niche and differ in MM cases compared to normal BM. Protein synthesis mostly regulated at the initiation phase is critically important to the cells' phenotype, adaptation to changing conditions, and interface with their neighbors. Previously, we have demonstrated the critical role of translation initiation factors in MM cells and their modulation by microenvironmental cues. Thus, we conjectured that the MM-MSC interaction may affect translation initiation in both populations.

Objectives: We aimed to assess the effect of MM-MSCs proximity on cells phenotype. Furthermore, we appraised whether translation initiation factors eIF4E and eIF4G1's expression and targets are affected by proximity between BM-MSC and MM cells.

Methods: MSCs from BM samples of MM patients and healthy donors (ND) were co-cultured with MM cell line (U266) 72h after which the respective cell populations were harvested separately. MSCs and MM cells cultured alone served as controls. Cells were counted and assayed for death (trypan blue). Protein lysates were assayed for eIF4E and eIF4G1. We are currently assessing targets levels (c-Myc/cyclin-D1/NFkB/MMP9, and HIF1 α /SMAD5/ER) (Immunoblotting, zymogram) and completing experiments with a second MM cell line (ARPI).

Results: MM cell lines co-cultured with MM-MSCs displayed decreased total and dead cell counts (\downarrow 30-80%, $p < 0.05$), whereas MM-MSCs co-cultured with MM cell lines demonstrated a trend of elevated total and dead cell counts (\uparrow 30-200%, $p = 0.15$). U266 co-cultured with ND-MSCs showed diminished levels of eIF4E and eIF4G1 (\downarrow 40%, $p < 0.01$). MM cell lines co-cultured with MM-MSCs expressed elevated levels of both eIF4E and eIF4G1 (\uparrow 50%, $p < 0.05$). BM-MSCs (ND and MM) co-cultured with U266 showed increased expression of eIF4E and eIF4G1 (\uparrow 70-200%, $p < 0.05$).

Conclusions: These novel results demonstrate that crosstalk between MM cells and BM-MSCs affects translation initiation and introduce a new target for drug development. Moreover, these findings provide proof that translation initiation is an interface for the mutual transformation of one cell population by the other.

Poster Board Number: I57

UNPROVEN STEM CELL THERAPIES AND THERAPEUTIC FREEDOM: THE ITALIAN JOB

Blasimme, Alessandro

INSERM UMR 1027, Université de Toulouse 3 - Paul Sabatier, Faculté de Médecine, INSERM, Toulouse, France

The idea that adult stem cells are ethically "safer" than cells of embryonic or foetal origin inadvertently gained credit both in professional circles and in the public discourse. However, recent public controversies over the use of adult stem cells testify that while

the moral status of the embryo is no longer the main, nor the most pressing ethical issue on the table, the use of adult stem cells for therapeutic purposes presents no less daunting ethical stakes. New issues are taking shape at the translational frontier of stem cell science, one that both scientists and bioethicists need to pay closer attention to. As stem cells move closer to patients, the ethical analysis should finally direct its attention to the stakes that characterise this translational phase. This, especially in the light of emerging controversies, will help channelling innovation towards socially desirable outcomes and will thus contribute meaningfully to the development of a promising field of innovation.

In this paper, I reconstruct and analyse the case of an Italian organisation called Stamina Foundation that, despite lack of evidence about the safety and efficacy of its activity, provides unproven stem cell therapies under the so-called compassionate use framework. The case recently made headlines in Italy as well as in international scientific journals such as Nature, Science and The EMBO Journal and gave rise to fierce public debates, disputable judicial decisions and dedicated legislative initiatives by the Italian Government and by the Parliament. During the public discussion regarding this case, however, the ethical terms of the issue remained elusive: they therefore need to be unpacked and clarified. The emerging consensus in bioethics is that the commercial interests of the providers of unproven stem cell therapies configure for patients the risk of fraud and deception: this led to a growing body of scholarly contributions and position statements by scientific societies including the ISSCR. In the Italian case, however, the alleged treatments are now being reimbursed by the State: this novelty calls for our analyses to be able to look even deeper into the ethical issues at stake with unproven cell therapies. In particular, to advance the debate in the direction that is solicited by current controversies, in this paper I analyse - and indeed criticise on ethical grounds - the claims that underpin the Stamina method and the public decisions taken to cope with this controversial case, especially as far as the ethically sensible boundary between therapeutic freedom and state powers to regulate science and health care are concerned. Public controversies of this sort offer valuable opportunities for unpacking and discussing the assumptions that support different visions of regenerative medicine. In this domain scientists and bioethicists will have to collaborate to make sense of the trajectory of regenerative medicine. This is going to be crucial to promote intelligent debate, anticipate future controversies and steer the clinical translation of stem cell science towards socially and ethically responsible developments.

Poster Board Number: I58

EXPRESSION OF HSA-MIR-3065-3P WAS SIGNIFICANTLY DOWNREGULATED IN FANCONI ANEMIA PATIENT BONE MARROW DERIVED MESENCHYMAL STEM CELLS FOLLOWING BONE MARROW TRANSPLANTATION

Cagnan, Ilgin¹, Konu, Ozlen², Aerts Kaya, Fatima S.¹, Kuskonmaz, Baris³, Uckan Cetinkaya, Duygu¹, Gunel-Ozcan, Aysen¹

¹Institute of Health Sciences, Department of Stem Cell Sciences, Center for Stem Cell Research and Development, Hacettepe University, Ankara, Turkey, ²Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey, ³Pediatric Hematology, Bone Marrow Transplantation Unit, Hacettepe University Childrens Hospital, Ankara, Turkey

Fanconi anemia (FA) is an inherited disease having a high incidence of cancer predisposition, hematopoietic defects and developmental dysmorphisms. In this study, microRNA (miRNA) expression profile of bone marrow derived mesenchymal stem/stromal cells (BM-MSCs) of FA patients prior to bone marrow transplantation (pre-BMT) were compared with those obtained following BMT (post-BMT). FA pre-BMT MSCs (n=6) and FA post-BMT MSCs (n=6) were expanded in vitro and characterized for their cell surface markers and differentiation capacity to adipogenic and osteogenic lineages. The miRNA expression profile was performed using Affymetrix GeneChip 2.0 Array. Following RMA normalization, data analysis was carried out

in Partek Genomics Suite software package (Partek Incorporated). Principal Component Analysis and hierarchical clustering were performed. Differentially expressed miRNAs between pre-BMT and post-BMT groups were determined ($P < 0.05$). There were statistically significant differences in the expression of multiple noncoding RNAs between two groups such that expression of hsa-miR-3065-3p ($P < 0.001$) was downregulated in FA post-BMT MSCs when compared to pre-BMT samples. This Project is supported by The Scientific and Technological Research Council of Turkey (TUBITAK; project no: 110S021) in conjunction with EU COST action BM0805.

Poster Board Number: 159

XENO-FREE CHONDROGENESIS OF BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS - TOWARDS IMPROVED SOLUTIONS FOR CELL-BASED ARTICULAR CARTILAGE REPAIR

Skog, Maria¹, Muhonen, Virpi², Nystedt, Johanna³, Narcisi, Roberto⁴, Kontturi, Leena-Stiina⁵, Urtti, Arto⁵, Korhonen, Matti³, van Osch, Gerjo J.V.M.⁶, Kiviranta, Ilkka²

¹Department of Biosciences, University of Helsinki, Helsinki, Finland, ²Department of Orthopaedics and Traumatology, Hospital District of Helsinki and Uusimaa, Helsinki, Finland, ³Advanced Therapies and Product Development, Finnish Red Cross Blood Service, Helsinki, Finland, ⁴Department of Orthopedics, University Medical Center Rotterdam, Rotterdam, Netherlands, ⁵Centre for Drug Research, University of Helsinki, Helsinki, Finland, ⁶Department of Orthopedics and Department of Otorhinolaryngology, University Medical Center Rotterdam, Rotterdam, Netherlands

Introduction: Bone marrow-derived mesenchymal stromal cells (BM-MSCs) are a fascinating alternative cell source for cell-based articular cartilage repair. Human articular cartilage is highly specialized type of connective tissue that covers the ends of long bones enabling joint function. Due to its avascular and alymphatic nature articular cartilage has very limited capacity for self-repair; and defects left untreated often produce significant musculoskeletal morbidity. Current cell-based repair techniques use autologous articular cartilage biopsy as a cell source; however, this method entails many disadvantages, such as restrained quantity of biopsy material, donor site morbidity, dedifferentiation of chondrocytes during in vitro expansion, and mixed phenotype of the repair tissue. Hence, the need for a substitutive cell source is highly relevant. BM-MSCs could be the solution due to their inherent capacity for chondrogenic differentiation. Nevertheless, we are still in infancy regarding their use in cartilage repair. Few preclinical and clinical studies have been performed, and although MSCs have been reported to be as effective as autologous chondrocytes in cartilage repair; no reliable production of articular cartilage-like repair tissue has been accomplished. Chondrogenic treatment of the cells prior to transplantation could be one possible solution to enhance their regenerative capacity in vivo, however, a clinical-grade xeno-free differentiation method is lacking.

Materials and Methods: We developed a novel xeno-free chondrogenic differentiation medium and tested its functionality in vitro with BM-MSCs from three donors and three other cell types holding potential for articular cartilage repair. In addition, the effectiveness of the medium was compared to a conventional chondrogenic differentiation medium and a commercially available chondrogenic differentiation kit. **Results:** BM-MSCs cultured under xeno-free chondrogenic condition produced cartilaginous proteoglycan-rich extracellular matrix morphologically and structurally comparable to that of BM-MSCs cultured under conventional chondrogenic medium. In addition, chondrogenic marker genes were upregulated under both conditions, but significantly higher levels of SOX9, COL2A1, and ACAN were detected from the xeno-free BM-MSCs. Furthermore, xeno-free chondrogenic medium induced chondrogenesis in BM-MSCs at least as efficiently as in chondrocytes. Also, under xeno-free condition BM-MSCs produced collagen-rich extracellular matrix structurally corresponding to that of chondrocytes.

Conclusion: For truly clinical-grade cell production xeno-free conditions should span from the MSC isolation and expansion to the differentiation process because use of animal-derived cell culture reagents increase the risk of zoonoses and host immune reactions. In addition, xeno-antigens have been shown to induce alterations in crucial stem cell-specific signaling systems that maintain the stemness of MSCs. Therefore, we developed a characterized and xeno-free chondrogenic differentiation condition for BM-MSCs that can be adapted to clinical-grade cell production.

Poster Board Number: 160

MSC REGENERATIVE POTENTIAL: DOES DONOR AGE MATTER?

Arango, Martha L., Conget, Paulette

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 1×10^6) or two doses of medium conditioned by MSCs (1x or 6x) were administered intradermally 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 granulation tissue formation) and microscopically (re-epithelialization, dermal-epidermal junction formation, leukocyte infiltration, pattern of collagen deposition, fibrosis and skin appendage structure regeneration).

As expected, the kinetic of 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). We found that MSCs and their conditioned medium obtained from middle and elder donors were the more efficient tools to fasten wound closure (50% at day 4-5 for middle and elder vs. 6-8 for young). This difference was not overcome by increasing cell or medium dose. Furthermore, the administration of MSCs or their conditioned medium obtained from middle or elder donors restrained leucocyte infiltration and fibrosis, 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.

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Poster Board Number: 161

INTRA-ARTICULAR TRANSPLANTATION OF MESENCHYMAL STEM CELLS IN PATIENTS WITH KNEE OSTEOARTHRITIS CLINICAL TRIAL: PHASE I

Moghminasr, Reza, Aghdami, Nasser

Stem Cells and Developmental Biology, Royan Institute, Tehran, Islamic Republic of Iran

Introduction: The aim of this research is to determine safety of a single local autologous mesenchymal stem cells (MSCs) injection in patients with knee osteoarthritis at least six months duration unresponsive to nonsurgical treatment. The hypothesis of these articles is that the autologous MSCs injection would stimulate the healing process manifested by reduction in pain and also increase Range of Motion (ROM) and cartilage resurfacing of knee joint. The main conservative

treatment of this disease is based on reducing inflammation with rest, Non Steroidal Anti Inflammatory Drugs (NSAIDs), and physical therapy and knee replacement. MSCs can produce a number of growth factors and cytokines, TGF- β 1, VEGF, IL-10, that are important for tissue repair and remodeling. These growth factors have been shown to play a significant role in the repair. This phenomenon can act as the regeneration or healing.

Materials and Methods: Six patients received a single local injection of autologous MSCs. Three different methods were used to evaluate the safety and efficacy of them. The first criteria were evaluation of adverse event like edema swelling limitation of motion range and infection in clinical Examination and patients walking distance. The second criteria were WOMAC questionnaires. These indexes evaluated different items such as pain score, motion, stability, daily function, stiffness. The quantity criteria for these items were determined between (0 to 4). The third criteria were Visual Analogue Scale (VAS) for pain. The quantity criteria for VAS items were determined between (0 to 10). These third criteria evaluated on 1, 2, 6 and 12 months after injections of autologous MSCs.

Results: No adverse events were seen in this study. Significant decreases of pain were observed at 12 month follow up. However, pain did not totally resolve in all patients. The results were indicated that VAS criteria gradually decreased specially in 12 months after treatment and also similar results were determined for WOMAC criteria.

Discussion: The results of this study suggest that MSCs injection is safe and repeated MSCs injection can relieve pain and improve function in patients with long term knee osteoarthritis who had failed other treatments.

Poster Board Number: 162

PRENATAL ORGANOPHOSPHATE-INDUCED DEFICITS IN NEUROGENESIS WERE REVERSED BY MESENCHYMAL STEM CELLS IN A NOVEL AVIAN MODEL

Pinkas, Adi¹, Turgeman, Gadi², Yanai, Joseph¹

¹Hebrew University, Jerusalem, Israel, ²Ariel University, Ariel, Israel

A chick model for the mechanisms of neurobehavioral teratogenicity was established in our laboratories. Prehatch exposure to various agents, including chlorpyrifos, induced posthatch behavioral deficits and concomitant alterations in cholinergic receptor induced activation/translocation PKC γ . Progress in research on stem cell therapy can be accelerated if a simple model is provided. Toward this end, we are developing a simple chick model for the therapy of chlorpyrifos-induced defects in neurogenesis employing mesenchymal stem cells (MSC) which are easy to obtain, known to promote neurogenesis, and enable autologous transplantation.

Fertile chicken eggs were injected with 10 mg/kg chlorpyrifos on incubation day (E) 0 and 5. On E13 Dil-labeled MSC (approximately 200,000 cells suspended in 100 μ l PBS) were transplanted into blood vessels attached to the chorio-allantoic membrane below the air cell (blunt end) of the control and chlorpyrifos-exposed eggs. The MSC were isolated from the femur and tibia bones of day-old chicks and expanded in culture. Twenty four hours after hatching the brains were removed, cut frozen and stained immunohistochemically for doublecortin for the evaluation of neurogenesis in the area of the lateral striatum. Lateral striatum area of similarly treated groups was taken for qPCR analysis of neurogenesis-related genes.

Chicks with prehatch exposure to chlorpyrifos exhibited impaired neurogenesis as ascertained by reduction in doublecortin immunoreactive cells as compared to controls ($44 \pm 10\%$, $p < 0.001$); MSC transplantation completely reversed this deficit. Among the neurogenesis-related genes studied, the expression of BDNF, DCX, MAP2, and SOX2, was reduced by 23-33% ($p < 0.005$), whereas the apparent reduction in FGF2 and VEGF did not reach statistical significance. Whether, as in the doublecortin study, the alterations in gene expression could be reversed by MSC transplantation, remains the objective of our current investigation.

Our simple (almost complimentary) chick model suggests that developmental chlorpyrifos-induced defects in neurogenesis could be reversed with the potential of clinically feasible MSC therapy.

Poster Board Number: 163

IS THERE ANY CELL SURFACE DIFFERENCE BETWEEN HUMAN MESENCHYMAL STEM CELLS FROM DIFFERENT SOURCES?

Lavazza, Cristiana¹, Crosti, Mariacristina², Boldrin, Valentina¹, Parazzi, Valentina¹, Montemurro, Tiziana¹, Montelatici, Elisa¹, Giordano, Rosaria¹, Moro, Monica¹, Lazzari, Lorenza¹

¹Cell Factory, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy, ²Istituto Nazionale di Genetica Molecolare, Milano, Italy

Currently mesenchymal stem cells (MSC) are efficiently isolated from several cell sources but unfortunately a common and shared consensus on their phenotypic identity is still lacking due to different laboratory approaches in culturing and characterizing these cells.

The particular relevance of this study was the availability in our laboratory of 8 MSC sources (5 perinatal and 3 adult), the same culture conditions (medium and FBS batch) and the extensive in blind immunophenotype analysis. The goal was to underline a common cell trait and to possibly discover any specific peculiarity.

MSC from perinatal (umbilical cord blood - CB, Wharton's jelly - WJ), amniotic fluid - AF, pre-term umbilical cord - PC, chorionic villi - CV) and adult (bone marrow - BM, adipose tissue - AD, dental pulp - DP) sources were expanded and their growth, clonogenic potential and immunophenotype (CD45, CD34, CD133, CD144, CD117, CD31, CD90, CD105, CD73, CD146, CD271, alpha-SMA, NG2, PDGFR, SSEA4) were analyzed. As control, fibroblasts were used.

Macroscopically a distinctive trait between adult and perinatal compartments was the morphology where perinatal MSC were smaller, "diamond-shaped" with shorter prolongations and a more compact cellular body than adult ones. Regarding the clonogenic potential expressed as colony forming unit - fibroblast (CFU-F), the perinatal MSC showed a lower frequency of CFU-F than the adult counterpart and as expected the initial CFU-F frequency diminished during passages as sign of stemness decrease.

Concerning the immunophenotype, CD45, CD34, CD133, CD144, CD117, CD31 were negative while CD73 and CD105 were expressed on 100% of the adult and perinatal MSC. Different from what expected was the CD90 expression since AFMSC and CVMSC contained high percentage of CD90 negative cells undermining the current meaning of this marker.

Regarding the perivascular markers such as CD146 and PDGFR, all the adult MSC expressed both of them. While in the perinatal compartment, all the cell sources showed CD146 expression but only the CBMSC displayed very high percentage of PDGFR.

Interestingly, SSEA4, an early embryonic glycolipid antigen well known to be present on BM MSC, was expressed on all the adult compartments though at low percentages; however, between perinatal sources, a subset of CBMSC, CVMSC and PCMSC was SSEA4 positive, while WJMSC and AFMSC were completely negative.

In conclusion, the well-known MSC markers such as CD73 and CD105 remained more stable during passages while the less "conventional" markers strongly changed and most of all within the perinatal compartments. Therefore based on these results an "on/off" expression that could characterize the MSC source was not found but differences in expression levels could help in defining tissue specific MSC signature.

Poster Board Number: I64**CELL THERAPY FOR HEREDITARY DEAFNESS WITH BONE MARROW MESENCHYMAL STEM CELL AND THE ACTIVATION OF STEM CELL HOMING**

Kamiya, Kazusaku¹, Karasawa, Keiko¹, Anzai, Takashi¹, Minowa, Osamu², Ikeda, Katsuhisa¹

¹Juntendo University, Tokyo, Japan, ²RIKEN BioResource Center, Tsukuba, Japan

Congenital deafness affects about 1 in 1000 children and more than half of them have genetic background such as Connexin26 (Cx26) gene mutation. The strategy to rescue such heredity deafness has not been developed yet. Recently, a number of clinical studies for cell therapy have been reported and clinically used for several intractable diseases. Inner ear cell therapy for sensorineural hearing loss also has been studied using some laboratory animals, although the successful reports for the hearing recovery accompanied with supplementation of the normal functional cells followed by tissue repair; recovery of the cellular/molecular functions were still few. Previously, we developed a novel animal model for acute sensorineural hearing loss due to fibrocyte dysfunction and performed cell therapy with bone marrow mesenchymal stem cells (MSC) as supplementation of cochlear fibrocytes functioning for cochlear ion transport. We injected MSC into the lateral semicircular canal and a number of these stem cells were then detected in the injured area in the lateral wall. The transplanted animals showed a significantly higher hearing recovery ratio than controls. Furthermore, we applied this strategy to Cx26-conditional knockout mice which we developed as a model for hereditary hearing loss and confirmed that our MSC transplantation was safety and useful for the replacement of the Cx26-mutated cochlear mesenchymal cells functioning for the ion transport. To enhance the efficiency, we analyzed the machinery of the stem cell induction to the targeted site in cochlea and found that monocyte chemoattractant protein 1 (MCP1: CCL2) and stromal cell-derived factor-1 (SDF-1: CXCL12) played important roles for this cell induction as stem cell homing factors. To enhance MSC invasion to cochlea tissue, we developed a novel transplant strategy by induction of MCP1 /SDF1 expression in host cochlear tissue and enhanced expression of their receptors, chemokine (C-C motif) receptor 2 (CCR2) and C-X-C chemokine receptor type 4 (CXCR4) in MSC. With this strategy, we induced efficient invasion of MSC to inner ear tissue and differentiation to form gap junctions with Cx26 among transplanted MSCs in Cx26-deficient mouse inner ear.

Poster Board Number: I65**THE INFLUENCE OF HYPOXIA AND HEAT SHOCK ON THE CYTOKINE/CHEMOKINE SECRETION PROFILES IN MESENCHYMAL STROMAL CELLS DERIVED FROM HUMAN EXFOLIATED DECIDUOUS TEETH**

Pivoriunas, Augustas, Tunaitis, Virginijus, Jarmalaviciute, Akvile, Venalis, Algirdas

Department of Stem Cell Biology, State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania

New findings demonstrate the importance of paracrine mechanisms in the therapeutic action of human mesenchymal stromal cells (MSCs). Several studies have demonstrated that MSCs secrete factors with anti-inflammatory, antiapoptotic and trophic activities. Therefore, it has been proposed to use factors produced by MSCs for therapeutic purposes. The main advantage of this approach is that it does not require direct use of cells. However, this area is poorly investigated, in particular, more information is needed about the influence of stress (heat shock, hypoxia, etc.) on MSC secretome under serum-free culture conditions. In our experiments we have used MSCs derived from human exfoliated deciduous teeth. Cellular supernatants were collected from cells cultivated under normal conditions, heat shock (42°C for 60 min) and hypoxia (5% O₂ for 72 h). Then we have used multi-analyte ELISA kits for simultaneous profiling of multiple cytokines (IL1A, IL1B, IL2, IL4, IL6, IL8, IL10, IL12, IL17A, GM-CSF, TNF α ,

IFN γ) and chemokines (MCP-1, RANTES, MIP-1a, MIP-1b, IP-10, I-TAC, MIG, eotaxin, TARC, MDC, GRO α). Our results show that under serum-free culture conditions MSCs derived from human exfoliated deciduous teeth secrete considerable amounts of IL6, IL8, GM-CSF, MCP1, RANTES, IP-10 and GRO α . Interestingly, from all cytokines and chemokines tested only levels of IL6 and IP-10 were affected by stress. The levels of IL6 and IP-10 were downregulated by 50 % and 27 % under hypoxia, while heat shock induced only downregulation of IL6 by 28 %.

Thus, we identified some of the cytokines and chemokines secreted by MSCs derived from human exfoliated deciduous teeth and grown under serum-free culture conditions. We also show, that under hypoxic and thermal stresses MSCs can keep steady levels of IL8, GM-CSF, MCP1, RANTES and GRO α . By contrast, hypoxia induces significant downregulation of IL6 and IP-10.

These results may be useful for further studies of paracrine mechanisms involved in the therapeutic actions of MSCs.

Poster Board Number: I66**BIO SAFETY EVIDENCE: NO HARMFUL EFFECT OF LOCAL TRANSPLANTATION OF ALLOGENEIC MSC IN ORAL SQUAMOUS CELL CARCINOMA AT DYSPLASIA STAGE**

Plaza-Flores, Anita¹, Arango, Martha², Bruna, Flavia², Espinoza, Iris², Conget, Paulette²

¹Programa de Doctorado de Ciencias Médicas, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile, ²Instituto de Ciencias, Facultad de Medicina Clínica Alemana Universidad del Desarrollo, Santiago, Chile

Mesenchymal stromal cells, also referred as to mesenchymal stem cells (MSCs), are being envisioned as a tool for the treatment of several diseases. Although preliminary results are promising, transfer of MSC-based therapy to clinical practice depends, among others, on the generation of empirical data regarding their biosafety. In particular, the role of MSCs in carcinogenesis. While some researchers have shown that MSCs increase tumor malignancy, others suggest that they suppress it. To date, no data are available concerning the impact of MSC transplantation on the natural evolution of precancerous lesions. The aim of the present study is to determine whether local transplantation of allogeneic MSCs modify the progression of precancerous lesions at the stage of dysplasia, in an animal model that shares the etiology, the pathogenesis and the progression (hyperplasia-dysplasia-papilloma-carcinoma) of human precancerous lesions. Oral squamous cell carcinoma (OSCC) was induced in the buccal pouch of Syrian golden hamster by topical administration of the mutagen dimethylbenzanthracene. When dysplasia was evidenced (leukoplakia resolved, mucosa thickening, increased vascularization, no exophytic tissue), vehicle or 1×10^6 allogeneic MSCs were injected into the oral mucosa. Three and six weeks later, macro and microscopic analyses were performed. All the animals that received the vehicle developed papillomas (17/17; volume: mean 7.9, range 1.3-46.5 mm³) that progressed to carcinoma (17/17; volume: mean 79.0, range 3.2-182.1 mm³). In contrast, at the end of the study period, the animals that received MSCs presented dysplastic lesions (1/4) or small papillomas (3/4; volume: mean 4.3, range 1.6-8.8 mm³). Our preliminary data suggest that local administration of allogeneic MSCs not only is unharmed for individuals with OSCC but also prevent the progression of dysplastic precancerous lesions. Hence, they represent empirical evidence regarding the biosafety of donor MSCs and also on their putative therapeutic potential for the management of OSCC precancerous lesions.

Poster Board Number:167**BASAL EXPRESSION LEVELS OF PREGNANE X RECEPTOR AND GLUCOCORTICOID RECEPTOR ARE TWO KEY DETERMINANTS FOR THE BASAL AND TRANSCRIPTION FACTOR-INDUCED CYP3A4 EXPRESSION IN HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS AND ADIPOSE-DERIVED STEM CELLS****Chiang, Tsai-Shin, Lee, Hsuan-Shu***Institute of Biotechnology, Taipei, Taiwan*

A prediction system with high levels of cytochrome P450 (CYP) activities is beneficial for developing drugs to foresee potential drug-induced liver damage. Our previous studies have reported that human dermal fibroblasts could be successfully induced to express high levels of CYPs by delivery of 10 defined transcriptional regulators (10F). In this study, the 10F-induced expression and activities of CYPs in bone marrow-derived mesenchymal stem cells (BMMSCs) and adipose-derived stem cells (ADSCs) were evaluated. In addition, the basal expression levels of glucocorticoid receptor (gr), pregnane X receptor (pxr), hepatocyte nuclear factor (hnf) 4 α , hydrocarbon receptor nuclear translocator (arnt), and cyp3a4 were also determined. The results showed that the CYP activities could be induced by delivery of the 10F in the 2 types of cells. Moreover, the basal level of cyp3a4 not only better correlated with basal level of pxr ($R^2=0.9678$) and gr ($R^2=0.9534$) than arnt ($R^2=0.4877$) and hnf 4 α ($R^2=0.1841$), but also correlated with 10F-induced level of cyp3a4 ($R^2=0.7979$). Furthermore, the 10F-induced cyp3a4 level was highly correlated with basal level of pxr ($R^2=0.7805$), but not with the level of induced pxr ($R^2=0.2233$). These findings indicated that the basal levels of pxr and gr significantly determine the 10F-induced cyp3a4 expression in BMMSCs and ADSCs.

Poster Board Number:168**ISOLATION AND CHARACTERIZATION OF MESENCHYMAL STEM CELLS FROM CRYOPRESERVED CHORIONIC VILLI****Leoni, Giampaolo***R&D, Biocell Center, Busto Arsizio, Italy*

This study was designed to explore the potential of human fetal mesenchymal stem cells derived from chorionic villi (CV-MSC), in view of a possible applications for cellular therapy and regenerative medicine. Small amount (5mg) of native chorionic villi samples were GMP-cryopreserved. Following thawing and conventional enzymatic disaggregation, cells were in vitro cultured and analyzed for biological endpoints like cell viability, proliferation rate, immunophenotype, and differentiation potential. Maternal cell contamination was excluded by QF-PCR analysis. Genome stability, by karyotype analysis, genome-wide array-CGH and microsatellite analysis, were also explored. Immunophenotyping of cultured cells showed expression of typical MSC markers (CD44, CD105, CD73 and CD90) and absence of expression of hematopoietic markers (CD45, CD34, CD14, CD19 and HLA-DR). Analysis of multilineage potential showed efficient differentiation into adipocytes, osteocytes and chondrocytes. Karyotype and microsatellite analysis were performed until late culture passages. Preliminary data showed that the frequency of chromosomes aberrations at the different culture passages is not significantly different from the basal frequency found in primary culture and the microsatellites stability in human CV-MSC is maintained up to 20th in vitro culture passage. Data obtained from array CGH analysis comparing DNA from early to late passages did not show any copy number variations of DNA segments confirming that the in vitro culture did not induce any modification of the genome stability. Our findings indicate that it is possible to isolate and extensively expand MSC from CV and that the in vitro culture does not interfere with the DNA-repair systems since the DNA stability is maintained during in vitro expansion. Under these circumstances, CV-MSC could be suitable for therapeutic proposes.

Moreover the use of cell bank technology, on native samples, might represent a life-long available autologous cell source for perinatal or adult regenerative medicine.

Poster Board Number:170**HUMAN AMNIOTIC MEMBRANE: A PROMISING NATURAL BIOMATERIAL FOR TISSUE ENGINEERING****Wolbank, Susanne¹, Lindenmair, Andrea², Banerjee, Asmita², Nümberger, Sylvia², Hackl, Christa³, Hennerbichler, Simone³, Gabriel, Christian³, Eibl, Johann⁴, Redl, Heinz²**

¹Ludwig Boltzmann Institute for Traumatology/ Trauma Care Consult/ Austrian Cluster for Tissue Regeneration, Vienna/Linz, Austria, ²Ludwig Boltzmann Institute for Traumatology/ Austrian Cluster for Tissue Regeneration, Vienna/Linz, Austria, ³Red Cross Blood Transfusion Service of Upper Austria/ Austrian Cluster for Tissue Regeneration, Linz, Austria, ⁴Bio-Products & Bio-Engineering AG, Vienna, Austria

Human amniotic membrane (hAM) represents an abundantly available biomaterial being applied in many surgical procedures. Furthermore, it is consistently gaining access to new areas in tissue engineering and regenerative medicine. For tissue engineering applications, cells are usually combined with a suitable carrier substrate or the so called cell sheet technology is exerted. We have recognized that hAM represents a natural, preformed sheet of stem cells. Hence, we studied a new approach for bone, cartilage and nerve regeneration via differentiation hAM in-toto including its sessile cells.

For this, hAM biopsies were cultured under conditions including either osteogenic, chondrogenic or Schwann cell-like differentiation. The resulting hAM was characterized for lineage-specific parameter including mineralization and calcium contents, (immuno)histochemical analysis, quantification of glycosaminoglycans (GAG), qRT PCR for specific mRNAs as well as ELISA for neurotrophic factors. All differentiation strategies resulted in upregulation of the analyzed lineage-specific markers. Under osteogenic conditions mineralization was observed and most of the cells expressed osteocalcin after 28 days. These results were confirmed by increased calcium-content and mRNA expression of specific osteogenic markers. GAG production was induced under chondrogenic conditions, especially within an induction medium containing FGF-2. These findings were further attended by quantification of the GAG content, and by upregulation of chondrogenic markers at mRNA level. When inducing a Schwann cell-like phenotype in hAM, levels of neurotrophic factors were increased, while the cells took up a glia-like morphology. Thus, these in-vitro results are promising steps towards a versatile use of living hAM as a natural biomaterial for tissue engineering.

Poster Board Number:171**NEW TARGETS FOR MSC-MEDIATED IMMUNOMODULATION IN MOUSE****Zanotti, Lucia¹, Soldani, Cristiana¹, Ploia, Cristina¹, Moalli, Federica², Dander, Erica³, D'Amico, Giovanna³, Sarukhan, Adelaida¹, Viola, Antonella¹**
¹Humanitas Clinical and Research Center, Rozzano, Italy, ²Theodor Kocher Institute, University of Bern, Bern, Switzerland, ³Centro Ricerca "M. Tettamanti," Clinica Pediatrica Università degli Studi di Milano Bicocca, Monza, Italy

Chronic inflammation is involved in the pathogenesis of several human diseases, such as atherosclerosis, allergy and autoimmune diseases. Several anti-inflammatory pharmacological drugs are currently available but with limited efficacy and deleterious long-term consequences. Long lasting tolerance induction may represent an effective resolution for these diseases and scientists have become interested in therapies based on cells with immunomodulatory activity. It has been demonstrated that Mesenchymal Stem Cells (MSCs) exert potent immunosuppressive effect both in vitro and in vivo, although the results of clinical trials are still disappointing and the biological mechanisms responsible for MSC effect remain poorly defined. The aim of our study is to obtain a deeper understanding on MSC immunomodulatory mechanisms in vivo.

By transplanting alginate-encapsulated MSCs, we have recently shown that their therapeutic effect is completely independent of migration capacity or cell to cell contacts. Using TCR transgenic mice, we have found that encapsulated MSCs hamper recruitment of leukocytes and dendritic cells into inflamed lymph nodes. We speculated that this could be due to a direct effect on endothelial activation. Here we show that, in vivo, MSC hamper the up-regulation of VCAM and the increase in the number of CD31 positive vessels in inflamed lymph nodes. Furthermore, MSC-conditioned medium is able to down regulate the expression of VCAM1 and ICAM1 on endothelial cell lines in vitro. Altogether, these data strongly suggest that soluble factors secreted by MSC can modulate immune responses by directly inhibiting endothelial cell activation. The precise molecules involved in this process are currently under investigation.

Poster Board Number: I72

IMMUNOMODULATORY EFFECT OF AN ACELLULAR SKELETAL MUSCLE SCAFFOLD TRANSPLANTED IN A DISCORDANT XENOTRANSPLANTATION MODEL

Fishman, Jonathan M.¹, Urbani, Luca¹, Lowdell, Mark W.¹, Ansari, Tahera², Burns, Alan¹, Sibbons, Paul², Wood, Kathryn J.³, Birchall, Martin A.⁴, De Coppi, Paolo¹

¹Surgery, UCL Institute of Child Health, London, United Kingdom, ²Surgery, Northwick Park Institute for Medical Research, London, United Kingdom, ³Surgery, Nuffield Department of Surgery, Oxford, United Kingdom, ⁴Surgery, UCL Ear Institute, London, United Kingdom

Natural acellular scaffolds, composed of natural extracellular matrix (ECM), form the basis of an emerging generation of tissue-engineered organ and tissue replacements that, together with the delivery of stem cells, are capable of transforming healthcare. Allogeneic, or xenogeneic, decellularized scaffolds require biocompatibility and absence of rejection. With this work we aimed at investigate the cell-mediated immune response towards decellularized scaffolds in vitro and in vivo. Skeletal muscle scaffolds were decellularized, characterized in vitro and xenotransplanted. The cellular immune response towards scaffolds was evaluated by immunohistochemistry and quantified stereologically. T-cell proliferation and cytokines, as assessed by flow cytometry, formed an in vitro surrogate marker and correlate of the in vivo host immune response towards the scaffold. Decellularized scaffolds were free of major histocompatibility complex (MHC) class I and II antigens and were found to exert anti-inflammatory and immunosuppressive effects, as evidenced by delayed biodegradation time in vivo, reduced sensitized T-cell proliferative activity in vitro, reduced IL-2, IFN γ and raised IL-10 levels in cell-culture supernatants. In addition, scaffold transplantation induced polarization of the macrophage response towards an M2-phenotype driving host responses away from a classical TH1-proinflammatory profile and appear to down-regulate T-cell xeno-responses and TH1 effector function. Importantly, acellular scaffold allowed in vitro culture and improved survival of donor-derived xenogeneic muscle progenitor cells at 2 and 4 weeks in vivo (ki67+ cells).

In conclusion, this study significantly adds to our previous data by demonstrating how the implanted scaffolds can modulate the immune response in several ways, thereby inhibiting rejection in a xenotransplantation model and preventing the rejection of donor-derived xenogeneic cells in vivo. These results have substantial implications for the future clinical application of tissue-engineered therapies.

Poster Board Number: I73

THE ROLE OF MONOCYTES IN THE IMMUNOREGULATORY FUNCTION OF MULTIPOTENT STROMAL CELLS

Roelofs, Helene, Melief, Sara, Schrama, Ellen, Geutskens, Sacha, Tiemessen, Machteld, Brugman, Martijn, Fibbe, Willem
Leiden University Medical Center, Leiden, Netherlands

Multipotent stromal cells (MSC) have already entered the clinical era

despite limited consensus on their mode of action. Many molecules have been appointed key factors for the immunomodulatory function of MSC. Accumulating evidence indicates that the immunosuppressive properties of MSC can be stimulated and that effective immunosuppression by MSC might require the involvement of accessory cells, especially monocytes/macrophages. In our studies we have focused on the interaction between MSC and monocytes.

Using in vitro assays for differentiation of monocytes and for generation of regulatory T cells (Treg), we have investigated the effect of MSC on monocytes. Using inhibition and depletion strategies we searched for cellular and molecular components that are essential in these interactions and for the functionality of MSCs. Further, we performed expression analysis by array technology on monocyte populations cultured in the absence or presence of MSC-derived factors.

We found that MSC inhibit the differentiation of monocytes towards dendritic cells and rather skew monocytes towards an IL-10 producing cell population with anti-inflammatory function. A key MSC-produced factor in this process is IL-6. The generation of Treg is markedly enhanced by the presence of MSCs and is critically dependent on the presence of monocytes. Also in this system the presence of MSCs enhances IL-10 secretion and skew monocytes towards a cell population with a macrophage type 2 phenotype. We show that, upon interaction with MSCs, monocytes upregulate CCL18, which proved to be essential for the observed Treg induction.

Improvement of knowledge on the mechanism of immunomodulation by MSCs supports the improvement of MSC therapy for immunologic or inflammatory diseases either by optimization of the environment in the patient or selection of patients based on more defined parameters, or by improvement of the therapeutic product, preparing MSCs ex vivo for their tasks in vivo, or by replacing MSCs altogether by a cocktail of factors they produce.

Poster Board Number: I74

CARDIAC EXTRACELLULAR MATRIX ENHANCES CARDIOMYOCYTE DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS (IPSC)

Fong, Ashley H.¹, Romero Lopez, Monica², George, Steven C.², Hughes, Christopher C.W.¹

¹Molecular Biology and Biochemistry, University of California, Irvine, Irvine, CA, USA,

²Biomedical Engineering, University of California, Irvine, Irvine, CA, USA

Cellular therapies using cardiomyocytes (CM) differentiated from pluripotent stem cells have great promise in treating myocardial infarction. Numerous protocols exist for differentiating stem cells into cardiomyocytes, mostly using soluble factors such as inhibitors, recombinant proteins or conditioned medium. However, extracellular matrix (ECM) proteins have been less well studied in the context of cardiac differentiation in monolayers. The majority of cardiac differentiation protocols that employ a specific substrate use Matrigel – an ECM derived from mouse Engelbreth-Holm-Swarm tumors, consisting primarily of laminin, collagen IV, and entactin. We hypothesized that the use of naturally-derived cardiac ECM would be a better substrate for cardiac differentiation than tumor ECM. We prepared cardiac ECM by SDS-mediated decellularization of bovine cardiac tissue, followed by lyophilization, resuspension and digestion with pepsin into a soluble scaffold. We then compared cardiac ECM and Matrigel for their ability to increase cardiac differentiation of human iPSC using Activin A and BMP4. We find that over a 21-day differentiation period, relative to Matrigel, cardiac ECM significantly increases the number of cardiac troponin T (cTNT)-positive cardiomyocytes as well as the intensity of the expression of Gata4 (4 fold), Myocyte-specific enhancer factor 2C (23 fold), Insulin gene enhancer protein 1 (11 fold), Tbx20 (5 fold), and cTNT (22 fold). Overall, the gene expression signature suggests that cardiac ECM improves cardiomyocyte differentiation compared to Matrigel. These studies suggest a protocol for improving cardiac differentiation that may yield both an increased number of cardiomyocytes as well as greater functionality.

Poster Board Number: 175**DISSECTING THE CARDIAC SCA1+ CELL BY CLONAL ANALYSIS, FATE-MAPPING, AND SINGLE-CELL GENE EXPRESSION: MOSAICISM OF KEY TRANSCRIPTION FACTORS AND CO-SEGREGATION WITH PDGFR-ALPHA**

Noseda, Michela¹, Harada, Mutsuo¹, McSweeney, Sara¹, Belian, Elisa¹, Leja, Thomas¹, Macaulay, Iain², Abreu Paiva, Marta S.¹, Al-Beidh, Farah¹, Sampson, Robert¹, Barahona, Mauricio³, Jacobsen, Sten Eirik², Schneider, Michael D.¹

¹British Heart Foundation Centre of Research Excellence, National Heart and Lung Institute, Imperial College London, London, United Kingdom, ²Haematopoietic Stem Cell Biology, MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom, ³British Heart Foundation Centre of Research Excellence, Department of Mathematics, Imperial College London, London, United Kingdom

Fate-mapping studies show that mammalian cardiac regeneration exists and occurs in part through a lineage decision by progenitor/stem cells. Several markers have been proposed for the identification of adult heart resident progenitors. Existing work has chiefly relied on purified but heterogeneous populations. Here, we dissected the cardiac Sca-1⁺ population through clonal analysis, fate-mapping and single-cell qRT-PCR. Clonogenicity after single-cell deposition was predicted by the side population (SP) phenotype. We report a library of directly cloned cardiac Sca-1⁺ SP cells that can be propagated for months, retain Sca-1 expression and SP phenotype (are self-renewing), are enriched for 2^o clone formation, and show tri-lineage potential (cardiac, endothelial, and smooth muscle) after grafting to the heart. The clones' molecular signature comprises stem cell markers plus many cardiac transcription factors, in the absence of cardiac structural genes or vascular markers. However, individual clones expressed incomplete subsets of the key factors Gata4, Mef2c, Tbx5 and Hand2. Single-cell qRT-PCR showed that fresh SP cells are likewise highly enriched for cardiac transcription factors, relative to non-SP cells, and confirmed the existence of micro-heterogeneities. By Cre/lox fate-mapping, cardiac SP cells derive from Mesp1-fated mesoderm, largely from precursors formerly expressing Nkx2-5 and Isl1, as well as cGata5, a marker of proepicardial origin. By single cell QRT-PCR, Pdgfra and Tcf21 correlated with the SP phenotype, but pinpointing cardiac transcription factors with better precision, even in non-SP cells. Unexpectedly, 15% of non-SP cells showed the molecular signature of SP cells and vice versa, which co-segregated with Pdgfra (and PDGFR α ⁺). PDGFR α ⁺ cells lacked these factors, expressed Kdr/Flk1, Cdh5, and CD31, and had less clonal growth, regardless of SP status. Thus: 1) cardiac Sca-1⁺ SP cells are clonogenic and have tri-lineage potential; 2) clones and single SP cells are enriched for heart-forming transcription factors, typically as incomplete mosaic patterns that may help prevent precocious differentiation; 3) cardiac Sca-1⁺ SP cells derive largely from Nkx2-5⁺ and Isl1⁺ cells, suggesting an origin only in part from the proepicardium; 4) PDGFR α distinguishes the SP and non-SP cells enriched for cardiac transcription factors (~85% and 15% respectively); 5) given the preponderance of non-SP cells in adult myocardium, PDGFR α ⁺ unmasks a more abundant source of cells enriched for cardiogenic genes than the use of SP cells alone.

Poster Board Number: 176**MODELING CARDIOMYOPATHY ASSOCIATED WITH A GATA4 MUTATION USING HUMAN INDUCED PLURIPOTENT STEM CELLS**

Rivas, Renee¹, Ang, Yen-Sin¹, Foley, Amy¹, Roach, Jared², Huff, Chad³, Hood, Leroy², Holloway, Alisha¹, Pollard, Katherine¹, Galas, David⁴, Srivastava, Deepak¹

¹Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA, ²Institute for Systems Biology, Seattle, WA, USA, ³University of Texas MD Anderson Cancer Center, Houston, TX, USA, ⁴Pacific Northwest Diabetes Research Institute, Seattle, WA, USA

Congenital heart disease (CHD) affects up to 0.5-1% of all newborns. Several families with CHD have been found with mutations in

transcription factors that are essential for cardiac development in mouse models. GATA4 is a transcription factor that is mutated in a families affected with atrial and ventricular septal defects (ASDs and VSDs). Despite surgical repair of septal defects, some family members with a GATA4 G296S mutation have developed a cardiomyopathy in their second decade of life. GATA4 heterozygous mice develop cardiomyopathy upon pressure-overload stress. We tested the hypothesis that mutation of a modifier gene in a subset of family members with the GATA4 G296S mutation results in cardiomyopathy. Complete genome sequencing of twenty-five members of the GATA4 G296S pedigree was performed and analyzed, yielding a candidate list of genetic modifiers for the cardiomyopathy phenotype. We used dermal fibroblasts from eight affected or unaffected related controls to generate integration-free iPS cells and differentiated them to cardiomyocytes. We are using iPS-derived cardiomyocytes to define the role of GATA4 in human cardiomyocytes and evaluate potential genetic modifiers of GATA4 using functional and genomic methods. We will present our results of this method of evaluating the genetic and cellular causes of cardiomyopathy and CHD in iPS cell-derived cardiomyocytes.

Poster Board Number: 177**IMPROVED EFFICIENCY OF DIRECT REPROGRAMMING TO CARDIOMYOCYTES FROM FIBROBLASTS WITH THE USE OF SMALL MOLECULES**

Ilkovits, Jamie L., Addis, Russell C., Gearhart, John D.

Institute for Regenerative Medicine & Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA, USA

Recent studies have been successful at utilizing overexpression of transcription factors to generate cardiomyocyte-like cells from fibroblasts, albeit at a low frequency in vitro. Although improved efficiency is observed in vivo following induction of MI in rodent models, additional work needs to be completed to generate up to 100M cardiomyocytes necessary to repair the human heart post-injury. This work focuses on identifying small molecules to enhance direct reprogramming towards cardiomyocytes. Fibroblasts were transduced with lentiviral vectors inducing expression of five transcription factors and a reporter construct in which the genetically-encoded calcium indicator GCaMP is driven by the cardiomyocyte-specific Troponin T promoter. The small molecules were added, alone or in combination, at different time points. Reprogramming efficiency was evaluated at Day 14 using both ICC of cardiomyocyte-specific structural proteins and the number of cells demonstrating robust calcium oscillations. Robust beating of single and groups of cells was observed in conditions treated with two different molecules as early as Day 10. Upon GCaMP quantification, ~3-5X the number cells displaying calcium transients were observed in the groups treated with the same two molecules compared to the vehicle control. Current work aims to investigate the mechanism behind the improved efficiency to facilitate the development of alternative treatment strategies for the clinic.

Poster Board Number: 179**THE ETIOLOGY OF DIABETES MODIFY THE RENO-PROTECTIVE EFFECT OF DONOR MULTIPOTENT MESENCHYMAL STROMAL CELLS?**

Ezquer, Fernando E.¹, Cabezas, Fabian A.², Giraud-Billoud, Maximiliano G.³, Carpio, Daniel J.⁴, Conget, Paulette A.¹, **Ezquer, Marcelo E.**¹

¹Instituto de Ciencias, Facultad de Medicina Clínica Alemana Universidad Del Desarrollo, Sanitago, Chile, ²Escuela de Ingeniería en Biotecnología, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Sanitago, Chile, ³Instituto de Fisiología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, IHEM-CONICET, Mendoza, Argentina, ⁴Instituto de Anatomía, Histología y Anatomía, Facultad de Medicina, Universidad Austral, Valdivia, Chile

The most detrimental complication of diabetes mellitus (DM) is diabetic nephropathy (DN), a clinical syndrome comprised of kidney damage and increased risk for cardiovascular disease. DN is associates

with functional and histological changes that include: albuminuria, mesangial expansion, glomerulosclerosis and tubulointerstitial fibrosis. Until now, there is no cure to DN; patient's management comprises the use of drugs to control hyperglycemia and blood pressure. If required, hemodialysis is prescribed. Unfortunately, these treatments only help to slow DN progression.

The progression of DN, secondary to type 1 DM (T1DM) or type 2 DM (T2DM), follows the same pathophysiological sequence. Nevertheless, in general the patients with T2DM have additional risk factors which exacerbate renal damage (hypertension, obesity, dyslipidemia and renal ischemic disease provoked by arteriosclerosis), making the management of this cases more complex than ND derived from T1DM.

Multipotent mesenchymal stromal cells also referred as mesenchymal stem cells (MSCs), are one of the most promising tool to manage DN progression, not only because they can be safely transplanted in human patients but also due to their proved renoprotective potential. The later, has been attributed to the capacity of MSCs to: i) reduce oxidative stress, ii) modulate chronic inflammation and macrophage infiltration, iii) avoid/revert fibrosis, iv) secrete trophic factors, and v) differentiate into pericytes, mesangial and tubular cells.

The aim of this work was to evaluate whether the MSC renoprotective effect depends on DM etiology (T1DM vs T2DM).

We used C57BL/6 mice rendered diabetic by the administration of a single high dose of streptozotocin, DN derived from T1DM (ND-T1DM) or C57BL/6 mice chronically exposed to high fat diet, DN derived from T2DM (DN-T2DM).

DN-T1DM and DN-T2DM animals received intravenously a single dose of MSCs (0.5x10⁶) at 8 and 25 weeks of diabetes respectively, since at this time diabetic mice were in an stage in which albuminuria was present, but most of the renal structures were still well preserved. Renal failure did not progress in MSC-treated mice, while in untreated mice albuminuria gradually increased in both animal models. These changes were correlated with morphological alterations including: mesangial expansion, podocyte loss, increased glomerulosclerosis index and tubular fibrosis. The observed renoprotective effects of MSC were not related to a reversion of the DM state.

In order to study the homing of MSCs in the kidney, donor cells were isolated from mice that constitutively express GFP in all their tissues (MSCGFP). The presence of MSCGFP was assessed in 2 and 8 weeks after administration, by flow cytometry and immunohistofluorescence. We found donor cells in the kidney of diabetic mice (type 1 and type 2), but not in normal mice.

Although donor cells were found in the kidney of treated mice, their scarcity suggests that the improvement in kidney function was not mediated by the differentiation of MSC into renal cells, so we suppose the participation of paracrine mechanisms to limit the progression of DN in MSC-treated mice.

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Poster Board Number:180

CARTILAGE TISSUE ENGINEERING USING PLURIPOTENT HUMAN EMBRYONIC STEM CELLS

Kimber, Susan J.¹, Cheng, Aixin¹, Griffiths, Rosie S.¹, Kapacee, Zoher¹, Ronshaugen, Matthew¹, Hardingham, Timothy E.²

¹Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom,

²Wellcome Trust Centre for Cell Matrix Research, Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom

Introduction: Cartilage is an avascular tissue which regenerate very poorly once damaged. Although autologous chondrocytes implantation (ATI) is one of the most successful methods to treat cartilage defects in clinic the limited cell resource and complexity of the procedure has limited the wide application of this in clinical practise. Embryonic stem cells (ESCs) are one of the most attractive cell types for tissue engineering due to their two distinct properties: unlimited self-renewal and pluripotency. With a view to developing human (h)ESC-derived chondrocytes for clinical application, we developed a protocol, using a

serum-free chemically defined medium, to generate chondroprogenitors from hESCs in vitro and test the ability of these cells for cartilage repair in vivo.

Methods: hESCs were initially cultured and expanded on feeder cells and then transferred to a feeder-free, serum-free culture system using fibronectin coated culture plates (Baxter et al., 2009 Stem Cell Research 3 28). iPSCs were generated from human dermal fibroblasts using retroviral reprogramming with OCT-4 SOX-2 KLF-4 AND CMYC. Chondrogenesis was initiated in hESC/iPSC by applying a sequential series of growth factors, which drive the hESCs through mesoderm and mesoderm to a chondrogenic cell type as previously reported (Oldershaw et al., 2010 Nature Biotech 28, 1187). The cells were characterised by quantitative RT-PCR using a range of chondrocyte-specific marker genes and immunostaining including for SOX9, the master transcription regulator for chondrogenesis. We also investigated progression from hESC to chondrogenic cells using RNASeq array technology. The cells were grown in pellet culture and in fibrin and other scaffolds with the aim of promoting their maturity. To test the cartilage formation capacity, these cells were implanted into to an osteochondral defect in the patella groove of Nude rats and assessed after different times, up to 12 weeks.

Results: Two/two iPSC and 4/5 hESC lines responded efficiently to the protocol generating chondroprogenitors. Chondroprogenitors derived from hESCs show typical properties of chondrocytes including, including high expression of SOX9, Collagen II and Collagen IX, XI and importantly a lack of Collagen X, an indicator for chondrocyte hypertrophy. Chondrogenic cells showed wide scale changes in micro-RNAs including down regulation of many associated with hESC. The chondroprogenitors could be cultured in a 3-dimensional fibrin gel and still retain their phenotype. When implanted into cartilage defects in Nude rats the hESC-chondroprogenitors formed cartilage, which at 12 weeks strongly stained with Safranin O and expressed Collagen II as well as still retaining human cells.

Conclusion: Our protocol using feeder free, serum free and chemical defined medium to induce chondrogenesis from hESCs is able to generate chondrocytes which can undertake cartilage repair in vivo. This protocol has potential to be developed for clinical applications.

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EQUINE INDUCED PLURIPOTENT STEM CELLS: DIFFERENTIATION INTO OSTEOGENIC AND CHONDROGENIC LINEAGES

Pinto, Filipa¹, Mason, Jeffrey², Pan, Wenyu¹, Richardson, Dean W.², Gearhart, John D.¹

¹Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA, ²New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA

Injuries in connective tissues occur at a high frequency in the horse as a consequence of strenuous activities, such as high profile races. Although, mesenchymal stem cells are currently the cells of choice in the treatment of these lesions, limitations due to the isolation and the number of cells obtained, require the search of a new source of cells, such as induced pluripotent stem cells (iPSCs). In this study we transduced adult dermal fibroblasts with a multicistronic vector expressing the transcription factors Oct4, Sox2, Klf4 and c-myc to generate Equine iPSCs (EqiPSCs). Colonies resembling embryonic stem-like cells were visible within a week after transduction and subsequently were picked and expanded for further characterization. Established EqiPSCs could be kept in culture for periods of at least 6 months, maintaining a stable karyotype and their morphological characteristics. We observed the expression of several markers associated with pluripotency in EqiPSCs, such as endogenous OCT4, NANOG, LIN28, SSEA1 and SSEA4. In addition, the EqiPSCs were able to form teratomas when injected in immunocompromised mice. These tumors comprised cells of all the three germ layers, as evidenced by the expression of Desmin and COL2A1 (mesoderm), SOX17 and GATA6 (endoderm) and Tubulin β 3 and KRT19 (neuroectoderm).

We then tested the ability of EqiPSCs to differentiate in vitro into more specific cell types with potential use in horse therapies. Using an osteogenic differentiation protocol we were able to obtain osteoclasts as determined by the von Kossa and Alizarin Red stainings. We then evaluated the ability of the EqiPSCs to differentiate into chondrocytes, by subjecting these pluripotent cells to micromass cultures in the presence of TGF- β 3. After 3 weeks of differentiation, the staining of the micromasses with Alcian Blue revealed the presence of acidic polysaccharides, a major component in mature cartilage. Real-time PCR analysis also showed increased expression of SOX9, COL2A1 and ACAN, known chondrogenic associated markers. Our results show that EqiPSCs can be successfully differentiated into cells of connective tissues, such as bone and cartilage cells, thus demonstrating the potential use of the EqiPSCs for regenerative medicine applications in the horse.

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FGF2 PRIMES PERIOSTEAL CELLS FOR ENDOCHONDRAL OSSIFICATION VIA MAINTENANCE OF SKELETAL PRECURSORS AND MODULATION OF BMP SIGNALING

van Gastel, Nick¹, Stegen, Steve¹, Van Looveren, Riet¹, Stockmans, Ingrid¹, Schrooten, Jan², Graf, Daniel³, Luyten, Frank P⁴, Carmeliet, Geert¹
¹Laboratory of Clinical and Experimental Endocrinology, KU Leuven, Leuven, Belgium, ²Department of Metallurgy and Materials Engineering, KU Leuven, Leuven, Belgium, ³Institute of Oral Biology, Center for Dental Medicine, University of Zurich, Zurich, Switzerland, ⁴Skeletal Biology and Engineering Research Center, KU Leuven, Leuven, Belgium

Cell-based tissue engineering may be a promising approach for the repair of large bone defects. The bone marrow has traditionally been the preferred cell source, but evidence suggests that periosteum represents a valuable alternative. Given the beneficial effects of fibroblast growth factor 2 (FGF2) on bone formation by bone marrow stromal cells and the potential of this factor to promote fracture healing, we hypothesized that FGF2 addition during culture expansion could enhance the bone-forming potential of murine periosteum-derived cells (mPDC).

The addition of FGF2 promoted mPDC proliferation and inhibited culture-induced senescence, confirming known effects of FGF2 on mesenchymal stem cells (MSC). When FGF2-pretreated mPDC (mPDC-FGF2) were implanted on a calcium phosphate-collagen carrier at an ectopic or orthotopic site in mice, a remarkable increase in newly formed bone, associated with large amounts of bone marrow, was observed after 8 weeks compared to control cells. Unexpectedly, mPDC-FGF2 also formed small islands of mature cartilage at both locations. To further investigate this aspect, we subcutaneously injected cells in collagen gels, which already after 1 week resulted in robust cartilage formation by mPDC-FGF2, but not by control mPDC. By 2 weeks this cartilage template underwent endochondral ossification, evidenced by the presence of invading blood vessels, chondroclasts and osteoblasts as well as mineralized matrix.

To elucidate the underlying mechanisms we assessed the number of skeletal precursors in the cultures. The mPDC-FGF2 populations contain significantly more Nestin⁺ MSC as well as osteoprogenitor cells, shown by increased Runx2 expression and a higher number of osteogenic colony-forming units. Surprisingly, the expression of chondrogenic lineage markers was reduced, indicating that the enhanced in vivo chondrogenesis of mPDC-FGF2 may be rather associated with the higher number of MSC. As we also detected a striking increase in BMP2 production by mPDC-FGF2, a factor with known osteochondrogenic potential, we genetically knocked down Bmp2 levels, resulting in a severe reduction of the endochondral bone-forming potential of the mPDC-FGF2.

Taken together, our results show that FGF2 markedly enhances the bone-forming potential of mPDC, providing new insights in the regulation of periosteal cells. As FGF2 levels are known to be increased during the early phases of fracture healing, it may there also

function as a trigger for periosteal progenitor cell expansion and, by augmenting BMP2 production by periosteal cells, prime the cells for subsequent osteogenic and chondrogenic differentiation. In addition, the use of FGF2-pretreated periosteal cells could pave the way for the development of an injectable cell-based system for the treatment of non-healing bone defects.

Poster Board Number: 183

GENERATION OF HIGHLY OSTEOGENIC BONE TISSUE ENGINEERED GRAFT FOR TREATMENT OF FRACTURE NON-UNION- PRECLINICAL TO PHASE I CLINICAL TRIAL

Teo, Erin Y.¹, Johana, Nuryanti B.¹, Chong, Mark SK², Tan, Lay Geok³, Chan, Jerry K.Y.¹

¹Department of Reproductive Medicine, KK Women's and Children's Hospital, Singapore, Singapore, ²School of Chemical and Biomedical Engineering, Division of Bioengineering, Nanyang Technological University, Singapore, Singapore, ³Department of Obstetrics and Gynaecology, National University of Singapore, Singapore, Singapore

Fracture has been a major clinical burden in Singapore, with up to 14,000 cases annually and non-union complicates around 10%. The current common treatment strategy for such cases is autologous bone grafting. However, autografts derived by implanting bone chips from a secondary site are often plagued with shortcomings such as donor-site morbidity, chronic pain and increased chances of infection. Studies have shown that a successful bone graft often includes synergistic interaction between the scaffold, cells and dynamic culture technology. In this study, a tissue engineered bone graft (TEBG) consisting of polycaprolactone (PCL) scaffold seeded with human fetal mesenchymal stem cells (hfMSC), primed using a bi-axial bioreactor has been developed. The FDA approved PCL scaffold has suitable degradation kinetics and its interconnected macroporous structure allows neo-vascularisation. hfMSC was selected for its high osteogenic capacity and lower immunogenicity as compared to perinatal and adult sources of MSC. In addition, our group has demonstrated in pre-clinical data that our TEBG conditioned in bi-axial bioreactor is highly capable and efficient in healing critical-sized femoral defect in mini-pigs. Mineralisation and eventual bridging was seen in a much earlier timepoint as compared to the controls of plain scaffold.

Following which, for the phase I trial, approximately 10 medically fit patients diagnosed with fracture of the tibia, femur, humerus, ulna or radius with bone loss resulting in non-union (gap type) for at least 6 months, with no significant pre-morbid medical history will be recruited in this trial. Patients will be offered the standard therapy of autologous bone grafting or demineralized bone matrix first, and will be recruited when these are refused. Baseline X-ray and high-resolution computer tomography (CT) scan will be used to determine the size and shape of the defect in 3D. The alloreactivity of the patients to the potential donor cGMP hfMSC samples will be evaluated through standard peripheral blood lymphocyte assays.

Sex-mismatched cGMP harvested and cultured hfMSC will be expanded to clinically relevant numbers and loaded onto the scaffold manufactured in accordance to patient's defect shape and size. The cell seeded construct will then be cultured for up to 14 days in the bioreactor. Biopsy of the TEBG will be done 12-24 hours prior transplantation for evaluation of its cellular viability, mineralisation and sterility.

Follow ups including post-operative blood test will be carried out (Day 0, 1, 7 and monthly up to 8 months), where lymphocytes selected through density centrifugation, and alloreactivity towards the donor hfMSC will be performed. Radiological follow ups using plain x-rays and CT will be performed monthly and yearly respectively to determine the bridging of fracture. Presence of donor cell migration from transplantation site will be detected through quantitative-fluorescent-PCR for specific STR sequences in female donor cells, in male patient's peripheral blood or opportunistic tissue biopsies in any future clinically indicated surgical operations. Follow ups will be carried out up to 2 years post-operation. Union of fracture will be defined as union of at

least 3 cortices as seen on two views of x-ray or complete bridging of fracture ends for 75% of cross sectional area of bone loss on CT imaging.

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CORD- BLOOD DERIVED UNRESTRICTED SOMATIC STEM CELLS: A PROMISING CANDIDATE FOR CELL- BASED THERAPY OF CIRRHOTIC LIVER ON EXPERIMENTAL LEVEL

Demerdash, Zeinab¹, EL Baz, Hanan¹, Kamel, Manal¹, Mohamed, Salwa¹, Mahmoud, Faten¹, Mansour, Wafaa¹, Hammam, Olfat², Mahmoud, Soheir³
¹Immunology, Theodor Bilharz Research Institute, Cairo, Egypt, ²Pathology, Theodor Bilharz Research Institute, Cairo, Egypt, ³Parasitology, Theodor Bilharz Research Institute, Cairo, Egypt

BACKGROUND AND AIM OF THE WORK: Cord blood (CB) derived Unrestricted Somatic Stem Cells (USSCs) with their multipotentiality hold great promise in liver regeneration. This work aims at evaluation of the therapeutic potentiality of USSCs in experimental model of CCl₄- induced liver fibrosis.

MATERIAL AND METHODS: Isolation, propagation, and characterization of USSCs from CB samples were performed. USSCs were induced to differentiate into osteoblasts, adipocytes, and hepatocyte-like cells. Twenty hamsters were induced to liver fibrosis by repeated intra peritoneal injection of CCl₄ for 8 weeks. The experimental model was designed as follows: 10 hamsters with liver fibrosis and treated with intra hepatic injection of 3x10⁶ USSCs (USSCs transplanted group), 10 hamsters with liver fibrosis (pathological control group), and 10 hamsters with healthy livers (normal control group). Animals were sacrificed 12 weeks after USSCs transplantation, and their liver sections were examined for detection of human hepatocyte-like cells by immunohistochemistry staining. Moreover, liver sections were examined for their fibrosis level, and their fibrotic indices were calculated. Sera of sacrificed animals were tested for liver functions.

RESULTS: CB USSCs, with fibroblast-like morphology, expressed high levels of CD44, CD90, CD73 and CD105 and were negative for CD34, CD45, and HLA-DR. USSCs showed high expression of transcripts for Oct4 and Sox2 and were in vitro differentiated into osteoblasts, adipocytes, and hepatocyte-like cells. In vitro induced hepatocyte-like cells were confirmed by cytoplasmic expression of glycogen, alpha fetoprotein, and cytokeratin 18. Animals were sacrificed 12 weeks after USSCs injection. Livers of USSCs transplanted group showed engraftment with human hepatocyte-like cells as proved by cytoplasmic expression of human alpha fetoprotein, cytokeratin 18, and OV6. In addition, livers of USSCs transplanted group showed less fibrosis than pathological control group. Liver functions in the form of serum AST & ALT level, and serum total bilirubin level were significantly lowered in USSCs transplanted group than pathological control group (p < 0.001). Moreover, the fibrotic index (percentage of fibrotic tissue in liver sections) was significantly lower (p < 0.001) in USSCs transplanted group than pathological control group.

CONCLUSIONS: On the basis of their ability to differentiate into osteoblasts, adipocytes, and hepatocyte-like cells, as well as their high expression of transcripts for Oct4, and Sox2, which are considered core transcription factors for pluripotency maintenance, CB-USSCs are introduced as multipotent stem cells with great potentiality in regenerative medicine. Our experimental liver fibrosis model transplanted with USSCs showed liver engraftment with human hepatocyte-like cells as well as signs of liver regeneration in the form of improvement in liver function assays and fibrosis level. The data presented here provide hope that human CB- derived USSCs could eventually be used in tissue replacement protocols for the treatment of inherited and acquired end-stage liver diseases. This project was supported financially by the Science and Technology Development Fund (STDF), Egypt, Grant No. 1410.

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THE THERAPEUTIC POTENTIAL OF HUMAN ADIPOSE TISSUE DERIVED MULTI-LINEAGE PROGENITOR CELLS IN THE TREATMENT OF LIVER FIBROSIS

Okura, Hanayuki¹, Soeda, Mayumi¹, Morita, Mitsuko¹, Senoo, Hachiro¹, Inui, Yoshiaki², Ichinose, Akihiro³, Kawata, Sumio², Matsuyama, Akifumi¹
¹Foundation for Biomedical Res & Innovation, Kobe, Japan, ²Hyogo Prefectural Nishinomiya Hospital, Nishinomiya, Japan, ³Kobe University Hospital, Kobe, Japan

Background: Liver fibrosis occurs in response to a variety of chronic injuries, including viral hepatitis, alcohol abuse, metabolic diseases, autoimmune attack of hepatocytes or the bile duct epithelium. Liver fibrosis is reversible, whereas cirrhosis, the end-stage result of fibrosis, generally is irreversible. Liver fibrosis is characterized by excessive accumulation of extracellular matrix, with the formation of scar tissue encapsulating the area of injury. Liver fibrosis has many clinical manifestations, including ascites, variceal hemorrhage, and encephalopathy. The prognosis for patients who have the disease is poor, although liver transplantation is a good alternative treatment. Limited numbers of donor livers, however, are available for the millions of patients who need them worldwide. Therefore, strategies involving exogenous cell replacement must be considered. Adult stem cells, which possess certain characteristics including self-renewal, proliferation, longevity, and differentiation, are a valuable source for transplantation. In a mouse model of liver failure, the systemic injection of bone marrow or bone marrow mesenchymal stem cells into mice rescues the diseased phenotype. Human adipose tissue-derived multi-lineage progenitor cells (hADMPCs) possess mesenchymal stem cell properties. The possibility of using hADMPCs to repair liver fibrosis, however, has not yet been evaluated. In this study, we investigated the therapeutic effect of hADMPC transplantation on carbon tetrachloride (CCl₄)-induced liver fibrosis in mice. **Methods:** hADMPC were isolated from subcutaneous adipose tissues of healthy volunteers and expanded in vitro, and identified by surface markers using flowcytometric analysis and by expression analysis of nuclear transcription factors (islet-1 and GATA-4) with RT-PCR. Male nude mice (body weight of 20-30 g) were treated with a mixture of CCl₄ (0.3mL/kg) and olive oil (1:1 vol/vol) by intra peritoneal injection twice a week for 5 weeks, and this was followed by a tail vein injection of hADMPCs (3x10⁷ cells/kg body, n=5) or placebo control (n=5), and followed by 5 more weeks of CCl₄ treatment. **Results:** After 5 more weeks of CCl₄ treatment (10 weeks in all), nude mice with hADMPC transplants [CCl₄ (10W)-hADMPC] exhibited a significant reduction in liver fibrosis, as evidenced by Sirius red staining in comparison with nude mice treated with CCl₄ for 10 weeks without hADMPC transplants [CCl₄ (10W)] (11% of fibrosis vs. 2.3%, respectively). Moreover, nude mice in the CCl₄ (10W)-hADMPC group had lower levels of serum glutamic oxaloacetic transaminase, glutamic pyruvate transaminase and Total bilirubin in comparison with the CCl₄ (10W) group (118.5 IU/L vs 155.8, 73.8 vs 122.6 IU/L, 0.060 vs 0.096 mg. dL, respectively). **Conclusion:** Our findings suggest that the systemic injection of hADMPCs is a novel approach for treating liver fibrosis and may be a promising therapeutic intervention in the future.

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THE THERAPEUTIC POTENTIAL OF ADIPOSE TISSUE DERIVED MULTI LINEAGE PROGENITOR CELL SHEETS IN THE TREATMENT OF LIVER FIBROSIS

Okura, Hanayuki¹, Soeda, Mayumi¹, Morita, Mitsuko¹, Senoo, Hachiro¹, Inui, Yoshiaki², Ichinose, Akihiro³, Kawata, Sumio², Matsuyama, Akifumi¹
¹Foundation for Biomedical Res & Innovation, Kobe, Japan, ²Hyogo Prefectural Nishinomiya Hospital, Nishinomiya, Japan, ³Kobe University Hospital, Kobe, Japan

Background: Liver fibrosis occurs in response to a variety of chronic injuries, including viral hepatitis, alcohol abuse, metabolic diseases, autoimmune attack of hepatocytes or the bile duct epithelium. Liver fibrosis is reversible, whereas cirrhosis, the end-stage result of fibrosis, generally is irreversible. Liver fibrosis is characterized by excessive

accumulation of extracellular matrix, with the formation of scar tissue encapsulating the area of injury. Liver fibrosis has many clinical manifestations, including ascites, variceal hemorrhage, and encephalopathy. The prognosis for patients who have the disease is poor, although liver transplantation is a good alternative treatment. Limited numbers of donor livers, however, are available for the millions of patients who need them worldwide. Therefore, strategies involving exogenous cell replacement must be considered. Adult stem cells, which possess certain characteristics including self-renewal, proliferation, longevity, and differentiation, are a valuable source for transplantation. In a rodent model of liver failure, the systemic injection of bone marrow or bone marrow mesenchymal stem cells into mice rescues the diseased phenotype. Adipose tissue-derived multi-lineage progenitor cells (ADMPCs) possess mesenchymal stem cell properties. The possibility of using ADMPCs to repair liver fibrosis, however, has not yet been evaluated. In this study, we investigated the therapeutic effect of ADMPC-sheet transplantation on carbon tetrachloride (CCl₄)-induced liver fibrosis in rats. Methods: Rat ADMPCs were isolated from inguinal adipose tissues of F344 male rats and expanded in vitro, and identified by surface markers using flowcytometric analysis. Eight-week-old female F344 rats (body weight of 120-130 g) were treated with a mixture of CCl₄ (0.3mL/kg) and olive oil (1:1 vol/vol) by intra peritoneal injection twice a week for 5 weeks, and this was followed by the transplantation of ADMPC-sheets (n=11) or mock operation (n=11), followed by 5 more weeks of CCl₄ treatment. Results: After 5 more weeks of CCl₄ treatment (10 weeks in all), rats with ADMPC-sheets transplants [CCl₄ (10W)-ADMPC-sheet] exhibited a significant reduction in liver fibrosis, as evidenced by Sirius red staining in comparison with nude mice treated with CCl₄ for 10 weeks without cell-sheet-transplants [CCl₄ (10W)] (22.2% of fibrosis vs. 10.8%, respectively). Moreover, rats in the CCl₄ (10W)-ADMPC-sheet group had higher levels of albumin (4.355 g/dL vs 4.273) and lower levels of serum glutamic oxaloacetic transaminase, glutamic pyruvate transaminase, alkaline phosphatase, gamma-GTP and total bilirubin the CCl₄ (10W) group (169.9IU/L vs. 190.7, 108.6 vs. 135.0 IU/L, 1292 vs. 1374, 8.9 vs. 15.5, 0.042 vs. 0.058 mg. dL, respectively). Conclusion: Our findings suggest that the systemic injection of ADMPC-sheet is a novel approach for treating liver fibrosis and may be a promising therapeutic intervention in the future.

Poster Board Number:187

TIMP GENES PLAY A ROLE IN THE MAINTENANCE OF MOUSE LIVER PROGENITOR CELL HOMEOSTASIS

Defamie, Virginie, Nissar, Aliyah A., Shao, Yang W., Khokha, Rama University of Toronto, Toronto, ON, Canada

Adult liver has the capacity for extensive regeneration in response to insult. In circumstances of acute liver failure and chronic liver disease, liver regeneration fails and liver transplantation is the only curative approach. However, the controlled manipulation of hepatic stem/progenitor cells and the specialized environment in which they reside, termed 'niche', holds great promise as an alternative therapy in currently untreatable liver diseases. The stem cell niche contains a complement of factors, supporting cells and extracellular matrix (ECM). TIMPs (tissue inhibitor of metalloproteinase) have been widely recognized to govern ECM integrity, cytokine and growth factor bio-availability, and are putative regulators for maintaining homeostasis of the stem cell niche. Tissue inhibitors of metalloproteinases TIMP1 and TIMP3 are also specific inhibitors of ADAM10 and ADAM17 which are involved in Notch activation. In the liver, Notch signaling is responsible for hepatic cell differentiation. This study uncovers liver niche alterations in *Timp1^{-/-}/Timp3^{-/-}* mice after various hepatic injury models which solicit progenitor cell activation. Results indicate an essential function of these TIMPs in liver development through regulation of Notch. The aberrant hepatic architecture in *Timp1^{-/-}/Timp3^{-/-}* results in a niche that affects liver stem/progenitor cell function capable of rescuing failed regeneration following 2/3 partial hepatectomy in *Timp3^{-/-}* alone. Taken together, these findings will contribute to a better understanding of the hepatic niche and may lead to treatments aimed at stimulation of a patient's own liver stem cell niche.

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TRANSPLANTATION OF ADIPOSE TISSUE DERIVED MULTI-LINEAGE PROGENITOR CELLS VIA PORTAL VEIN IMPROVED SERUM LEVELS OF BETA-GALACTOSIDASE IN GMI GANGLIOSIDOSIS MODEL MICE

Soeda, Mayumi¹, Okura, Hanayuki¹, Matsuda, Junichiro², Koura, Minako², Morita, Mitsuko¹, Ichinose, Akihiro³, Hayakawa, Takao⁴, Matsuyama, Akifumi¹ ¹Foundation for Biomedical Res & Innovation, Kobe, Japan, ²National Institute of Biomedical Innovation, Ibaraki, Japan, ³Kobe University hospital, Kobe, Japan, ⁴Pharmaceutical Research and Technology Institute, Kinki University, Higashi-Osaka, Japan

Background: GMI gangliosidosis and Morquio B are autosomal recessive storage disorders caused by the deficiency of β -galactosidase (GLBI). We have reported that adipose tissue-derived multi-lineage progenitor cells (ADMPCs) could be 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 GLBI levels of GMI-gangliosidosis model GLBI-knockout mice. Methods and Results: GLBI expressions of ADMPCs were examined by mannose-6-phosphate (M6P) and mannosamine competition release into cultured media. Incubation with them revealed GLBI secretion into cultured media in dose dependent manner; resulted in ADMPCs express GLBI. Next, GLBI-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 GLBI were examined by beta-galactosidase enzymatic activities. The serum levels of GLBI were 7.1 mU of beta-galactosidase (normal control mice, n=6), 0.46 (GLBI-knockout mice treated with lactic control, n=3) and 3.54 (GLBI-knockout mice treated with ADMPC, n=6), respectively. These results indicated the ADMPCs transplantation could improve GLBI deficiency in GMI-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 GMI-gangliosidosis.

Poster Board Number:201

SKIN TO BRAIN: JOURNEY OF INDUCED PLURIPOTENT STEM CELLS FROM SKIN TO FUNCTIONAL NEURONS

Sharma, Ruchi¹, Livesey, Matthew², Wyllie, David JA³, Hay, David⁴, Donadeu, Xavier¹

¹Developmental Biology, The Roslin Institute and Royal (Dick) School of Veterinary Studies, Edinburgh, United Kingdom, ²Centre for Integrative Physiology, University of Edinburgh, Hugh Robson Building, George square, Edinburgh, EH8 9XD, Edinburgh, United Kingdom, ³Centre for Integrative Physiology, University of Edinburgh, Hugh Robson Building, George square, EH8 9XD, Edinburgh, United Kingdom, ⁴MRC Centre for Regenerative Medicine, Edinburgh Bioquarter, 5 Little France Drive, Edinburgh, EH1 6 4UU, United Kingdom., Edinburgh, United Kingdom

Animal experimentation that has primarily been conducted on rodent and laboratory animals has contributed significantly in neurosciences both in human and veterinary medicine. But it is widely recognized that rodents are less predictable models as large mammalian brain differs not only in size but also in complexity in neuron coverage and synapses. Therefore, high fidelity models of neurogenesis in equines are required to advance anaesthesia research, understand neuropathies and neuronal conditions (equine motor neuron disease) similar to humans. But in contrast to humans, in vitro models of neurogenesis in equines do not exist. Therefore it has been necessary to explore the use of stem cells in constructing cell based equine models. Pluripotent stem cell populations are of great interest in this field given their ability to form 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 pluripotent stem cell populations.

Induced pluripotent stem cells (iPSCs) are generated by reprogramming somatic cells to an embryonic state. In this study, we describe the generation of iPSCs from equine skin cells and their efficient and robust differentiation into electrically active neurons with motor neuron like properties. The equine induced pluripotent stem cells (iPSCs) were generated from primary keratinocyte cultures transduced with retroviral constructs coding for murine Oct-4, Sox-2, c-Myc and Klf-4 as per original Yamanaka protocol. The iPSCs colonies had distinct sharp boundaries and stained positive for alkaline phosphatase resembling previously reported human iPSCs. The Equine iPSCs lines expressed pluripotency markers expressed in equine embryonic stages including, OCT4, SOX2, SSEA1, LIN 28, NANOG, REX1, TERT and DNMT3B. Equine iPSCs were able to differentiate into derivatives of the three germ layers both in vitro and in vivo with teratoma formation in SCID mice.

Equine iPSCs were successfully induced to differentiate into neurospheres forming extensive neuronal projections and synapses which expressed neuronal markers including Tuj-1, Map2, Synapsin I and Choline acetyl transferase (ChAT). Equine iPSCs were differentiated to motor neurons using a novel and robust approach. Differentiating cells expressed TUBB3 at induction before ISL1 up regulation, a potent and specific inducer of motor neurons, during terminal differentiation. Electrophysiology with whole cell current clamp recording established functionality. Neurons tested fired multiple action potentials that were then blocked by bath application of voltage-gated sodium ion channel blocker, tetrodotoxin.

We report for the first time the generation of electrically active equine neurons with motor neuron-like properties in vitro, providing, also for the first time demonstration of the potential of iPSCs in equine biomedicine. This constitutes novel and truly remarkable development in stem cell biology which can lead to exciting applications, enhance understanding of neurogenesis and development of in vitro models which is a major step towards achieving 2010/63/EU directive on 3Rs, replacement, reduction and refinement of use of animals in both human and animal research.

Poster Board Number:202

DERIVATION OF INDUCED PLURIPOTENT STEM CELLS AND DIFFERENTIATION TO SENSORY-LIKE NEURONS

Wilbrey, Anna, Nitzsche, Anja, Rosa, Maria Isabel, Stacey, Peter, Bilsland, James Neusentis, Pfizer, Cambridge, United Kingdom

Induced pluripotent stem cells (iPS) offer the opportunity to produce differentiated cell types directly from patient samples. This is particularly useful when the cell types of interest are difficult to obtain, such as sensory neurons to facilitate investigation of pain both in controls and disease conditions such as erythromelalgia. Previous work has shown that human embryonic stem cells can be differentiated into nociceptive neurons in vitro using a small molecule cocktail and we aimed to reproduce this using iPS cells. To this end, we derived iPS from human dermal fibroblasts (HDFs) using either lenti- or Sendai virus to deliver the Yamanaka reprogramming factors. These cells were shown to be pluripotent using immunocytochemistry and qPCR. They were then successfully differentiated into neurons that express sensory 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 (P2RX3 channel response) and KCl. In summary, we demonstrate derivation of iPS from HDFs and their ability to differentiate into sensory-like neurons.

Poster Board Number:203

IN VITRO DISEASE MODELING OF AMYOTROPHIC LATERAL SCLEROSIS WITH PATIENT-SPECIFIC IPS CELLS

Lenzi, Jessica¹, Riccardo de Santis², Rosa Alessandro² and Bozzoni Irene^{1,2}

¹Center for Life Nano Science at Sapienza, Istituto Italiano di Tecnologia, Sapienza University of Rome, Rome, Italy, ²Dept. of Biology and Biotechnology "C. Darwin", Sapienza University of Rome, Italy

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder characterized by the premature loss of upper and lower motor neurons. FUS/TLS (FUS) and TARDBP (encoding the protein TDP-43) are two genes associated to a subset of familial ALS cases. They are involved in multiple steps of RNA synthesis and processing including mRNA transcription, splicing, translation, transport and microRNA processing. However, their role in the onset of ALS is still unknown. The inability to sample human live brain cells limits the study of the molecular mechanisms underlying ALS pathogenesis. Adult somatic cells, such as skin fibroblasts, can be reprogrammed into induced pluripotent stem cells (iPSCs) using forced expression of the transcription factors Oct4, Sox2, Klf4 and c-Myc (reprogramming factors). From fibroblasts of ALS patients we have obtained iPSCs with mutations in FUS and TDP-43 that can be differentiated into genetically matched motor neurons with standard protocols. Since ALS is also a muscle disease, we have developed a protocol to differentiate our iPSCs in muscle fibers. Combining iPSC-derived motor neurons and muscle, we have established co-cultures of mutant and wild-type cell types. We are now taking advantage of this system to study the role of mutated FUS and TDP-43 proteins in ALS pathogenesis.

Poster Board Number:204

FUNCTIONAL ANALYSIS OF SOX6 IN HUMAN GLIOMAS AND GLIOMA STEM CELLS

Ohta, Shigeki¹, Fukaya, Raita², Thirant, Cécile³, Chneiweiss, Hérve³, Okano, Hideyuki¹, Kawakami, Yutaka⁴, Toda, Masahiro²

¹Physiology, Keio University School of Medicine, Tokyo, Japan, ²Neurosurgery, Keio University School of Medicine, Tokyo, Japan, ³Team Glial Plasticity Inserm UMR 894, University Paris Descartes, Paris, France, ⁴Institute for Advanced Medical Res., Keio University School of Medicine, Tokyo, Japan

The Sox6 gene is a member of the SOX gene family, which is characterized by transcription factors with a high-mobility-group (HMG) box DNA-binding domain highly similar to that of the sex-determining region (Sry) protein. In a previous study, we reported high expression of SOX6 in gliomas (Ueda R et al., *Oncogene* 23, 1420-1427, 2004). To date, high gene expression of Sox6 in brain tumors, including glioblastomas, is shown based on in silico analyses using DNA microarray databases similar to SOX2, SOX4, and SOX11, although the function of SOX6 in gliomas has remained largely unknown. In this study, we found high expression of the SOX6 gene transcript in glioma cell lines (KNS42, SF126) by quantitative real-time PCR. Intriguingly, gene silencing in the glioma cell lines using SOX6-specific siRNA decreased cell proliferation. In addition, we observed higher SOX6 gene expression in neural stem progenitor cells (NSPCs) derived from human fetal brains and glioma initiation cells (GICs), which were isolated from cancer patients, when compared to normal astrocytes. Treatment of GICs with lentivirally-expressed SOX6 shRNA resulted in reduced cell proliferation and cell cycle arrest, which were accompanied by an upregulation of p21, p27 and p53 gene expression. SOX6 gene silencing also induced apoptosis in GICs. Furthermore, gene silencing of SOX6 in GICs decreased primary sphere formation as seen in a murine embryonic NSPC culture system, accompanied with decreased SOX2 gene expression. Taken together, our results suggest that SOX6 may be a proliferation and/or survival factor of GICs, and a potential therapeutic target for the treatment of gliomas.

Poster Board Number:205**IDENTIFICATION OF CRITICAL FACTORS AFFECTING MICROGLIA RECONSTITUTION FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR DESIGNING THERAPEUTIC APPROACHES**

Capotondo, Alessia¹, Milazzo, Rita², Politi, Letterio Salvatore³, Cecere, Francesca¹, Nonis, Alessandro⁴, Di Serio, Clelia⁴, Montini, Eugenio¹, Naldini, Luigi², Biffi, Alessandra¹

¹Division of Regenerative Medicine, Stem Cells and Gene Therapy, HSR-TIGET, Milano, Italy, ²Division of Regenerative Medicine, Stem Cells and Gene Therapy, HSR-TIGET, Vita-Salute University, Milano, Italy, ³Imaging Core, Neuroradiology Unit, Head and Neck Department, San Raffaele Scientific Institute, Milano, Italy, ⁴Center of Biostatistics, Vita-Salute University, 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). However, obtaining microglia reconstitution 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. 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 as busulfan (BU) 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. In order to attempt characterizing the bona fide microglia progenitors ablated by the conditioning regimen 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 (ICV) delivery of GCV, we observed a variation of the composition of the myeloid brain compartment similar to what 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. Transplant experiments in this setting are on going for confirming this indication. Since we demonstrated that engraftment in the bone marrow might not be necessary for obtaining an efficient microglia reconstitution by the donor following HCT, these experiments will also contribute at better assessing the modalities of microglia reconstitution after ICV and/or intravenous HSPC transplantation. Importantly, we are working for the identification of the fraction within HSPC capable of short-term brain homing and local proliferation with the final goal of further optimizing transplant approaches. For this purpose, we are transplanting different sub-populations purified from the bone marrow HSPC pool. We showed that at 6 months post-transplant only KSL cells retain the capability of long-term microglia reconstitution. We are now working on 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. Finally, since recent work suggests that microglia emerge from yolk sac erythromyeloid progenitors, we are currently better characterizing these cells both in embryos and in adult mice, in the perspective of assessing their capability of microglia reconstitution upon transplantation in our preclinical models.

Poster Board Number:206**IGF-1 ACTIVATES A CILIUM-LOCALIZED NON-CANONICAL G β y SIGNALING PATHWAY THAT REGULATES CELL CYCLE PROGRESSION AND MOUSE NEURAL STEM CELL PROLIFERATION**

Sung, Ching-Hwa¹, Yeh, Celine¹, Li, Aiqun¹, Saito, Masaki¹, Caceres, Alfredo², Chuang, Jen-Zen¹

¹Ophthalmology, Weill Cornell Medical College, New York, NY, USA, ²INIMEC-CONICET, Cordoba, Argentina

Primary cilia are present on the surface of most G0/G1 cells, and are resorbed prior to cell cycle re-entry. Although it has been established that ciliary resorption and cell cycle re-entry are temporally coupled events, the environmental cues and molecular mechanisms that underlie this causal relationship are unknown. We previously demonstrated that the activation of phospho(T94)Tctex-1 at the ciliary transition zone is required for ciliary resorption and S phase re-entry, but is not necessary for the cell cycle progression of cells without cilia. This suggests that phospho(T94)Tctex-1 activation mediates the link between ciliary resorption and S phase re-entry. In the present study, we show that phospho(T94)Tctex-1 is a downstream effector of a novel, non-canonical G β y signaling pathway that is activated by IGF-1 receptor. Phospho(T94)Tctex-1 and phosphor-IGF-1 receptor are also selectively enriched at the ciliary transition zones of neural stem cells (NSC) in the neocortex. Perturbing any component of this pathway results in defective ciliary resorption, delays in S phase entry, and premature neuronal differentiation at the expense of NSC proliferation. Hence, our model suggests that ligand induced activation of IGF-1 receptor regulates the rate of ciliary resorption, which is a key determinant of the G1 length and fate specification of NSCs.

Poster Board Number:207**STEM CELL MEDIATED GENE THERAPY: STREAMLINED PREPARATION FOR CLINICAL TRIALS**

Metz, Marianne Z.¹, Tirughana-Sambandan, Revathiswari¹, Potter, Phillip M.², Synold, Timothy W.³, Hsu, David⁴, Ghoda, Lucy Y.⁵, Aboody, Karen S.⁶

¹Department of Neuroscience, Beckman Research Institute of City of Hope, Duarte, CA, USA, ²Department of Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, Memphis, TN, USA, ³Clinical Immunobiology Correlative Studies Laboratory, Beckman Research Institute of City of Hope, Duarte, CA, USA, ⁴Center for Biomedicine and Genetics, Beckman Research Institute of City of Hope, Duarte, CA, USA, ⁵Department of Neurosciences, Beckman Research Institute of City of Hope, Duarte, CA, USA, ⁶Department of Neurosciences, Division of Neurosurgery, Beckman Research Institute of City of Hope, Duarte, CA, USA

Human neural stem cells (NSCs) modified to express a therapeutic transgene, have been used for gene therapy in early phase I glioma clinical trials. NSCs hold great promise for glioma therapy due to their inherent tumor-tropic properties and use as vehicles for delivering chemotherapy directly to infiltrating glioma cells. HB1.F3.CD NSCs were further modified with a replication incompetent adenovirus containing a secretable modified human carboxylesterase gene, hCE1m6. This enzyme efficiently converts irinotecan (CPT-11) to its more potent active metabolite and topoisomerase inhibitor, SN-38, selectively killing dividing brain tumor cells in the vicinity of the NSCs, thus sparing normal tissues. Past protocols for NSC for patient use required a GMP facility, where frozen cells are thawed, plated, and modified for administration to the patient within 96 hours of thaw. Prior to infusion the NSCs must pass release tests under quality assurance (QA) and quality control (QC) FDA requirements. In order to perform a multi-center phase I clinical trial a more practical protocol is needed to streamline NSC preparation for patient administration. We have now developed protocols for adenovirally transducing the NSCs prior to freeze, with a simple thaw, wash, and resuspend preparation just prior to patient administration. Qualification runs on the frozen cell bank will be performed in advance of patient trial, so that only minimal testing is required on the day of patient administra-

tion following thaw and wash, without the need for a GMP facility. In the studies presented here, we compare the plated and freshly transduced NSCs vs. thawed frozen transduced NSCs (NSC.hCE1m6) cells for viability, identity, CE activity, and tumor-tropic properties. Data support the use of these novel freeze and preparation methods for CNS transplantation of virally transduced stem cells. This has significant impact on the practicality of multi-center use, and minimizes preparation costs.

Poster Board Number:208

HUMAN PARTHENOGENETIC STEM CELL BASED THERAPY IN PRECLINICAL MODELS OF PARKINSON'S DISEASE

Gonzalez, Rodolfo¹, Garitaonandia, Ibon¹, Crain, Andrew², Ostrowska, Alina¹, Abramihina, Tatiana¹, Wambua, Gerald¹, Noskov, Alexander¹, Poustovoitov, Maxim¹, Boscolo, Francesca³, Laurent, Louise³, Redmond, Jr, D. Eugene⁴, Snyder, Evan², **Semechkin, Ruslan¹**

¹International Stem Cell Corporation, Oceanside, CA, USA, ²Sanford-Burnham Medical Research Institute, La Jolla, CA, USA, ³UC San Diego, La Jolla, CA, USA, ⁴Yale University School of Medicine, New Haven, CT, USA

Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder affecting 5 million people worldwide. Pharmacological treatments are symptomatic and do not stop disease progression, have unwanted side effects, and become ineffective over time. Stem cell therapies can address this need and human parthenoetic stem cells (hpSCs) are a particularly good candidate because they differentiate into cells of all germ lineages, avoid the destruction of a potentially viable human embryo, and can be derived HLA-homozygous, making them histocompatible to significant segments of the human population. Here we report the generation of highly pure populations of neural stem cells (NSCs) and dopaminergic (DA) precursors from hpSCs through a chemically defined method and comparison of their efficacy in the treatment of rodent and non-human primate models of PD. In the rodent model, NSCs and DA precursors implanted in 6-OHDA-lesioned rats survived 28 weeks post-transplantation, successfully engrafted, and retained their neural phenotype with lack of overgrowth or tumors. Rats transplanted with both cell types significantly improved over sham control in the amphetamine-induced rotation test. In the non-human primate model, asymptomatic MPTP-lesioned African green monkeys with reduced dopamine levels in the nigrostriatal pathway, but no clinical symptoms were used to evaluate the safety of the therapy. Monkeys transplanted with both cell types had stable Parkinson's scores and healthy behavior four months post-transplantation with no adverse events such as dyskinesia or dystonia. We observed good survival and engraftment of both cell types with no signs of ectopic tissue or tumors. However, comparison of the dopamine levels in the striatum near the grafted sites indicated that NSCs were superior in restoring dopamine levels. We also observed higher TH+ DA neuron cell numbers in the substantia nigra and fiber innervation in the striatum of monkeys transplanted with NSCs. Additionally, the host immune response against the NSCs was much lower than DA precursors grafts, which were surrounded by reactive astrocytes and activated microglia. In conclusion, these results demonstrate for the first time functional benefits following transplantation of hpSC-derived NSCs and DA precursors in experimental models of PD.

Poster Board Number:209

RAT SPINAL CORD INJURY TREATMENT USING A COMBINATION OF HUMAN FETAL NEURAL STEM CELLS AND NOVEL HPMA-MOETACL HYDROGELS RELEASING CELL SUPPORTIVE MOLECULES

Růžička, Jiří¹, Romanyuk, Nataliya², Větrník, Miroslav³, Kárová, Kristýna⁴, Hrubý, Martin³, Price, Jack⁵, Syková, Eva⁴, Jendelová, Pavla⁴
¹Department of Neuroscience, 2nd Faculty of Medicine, Charles University, Prague- 5, Czech Republic, ²Department of Neuroscience, Institute of Experimental Medicine, ASCR, Prague 4 - Kř.č, Czech Republic, ³Institute of Macromolecular Chemistry, Prague, Czech Republic, ⁴Department of Neuroscience, Institute of Experimental Medicine, ASCR, Prague- 4, Czech Republic, ⁵Institute of Psychiatry, Kings College London, London, United Kingdom

Currently, there is no effective strategy for the treatment of spinal cord injury (SCI). A combination of stem cells and a hydrogel modified to effectively bridge the lesion cavity and support cell proliferation and differentiation is a promising approach to restore damaged spinal cord tissue and increase regenerative potential after SCI.

In our study we evaluated the growth, proliferation and distribution of immortalized human neural stem cells (line SPC-01 derived from fetal spinal cord tissue) in hydroxy propyl methacrylamid- [2-(methacryloyloxy)ethyl] trimethylammonium chloride (HPMA-MOETACL)-based hydrogels with the heterogenic ion exchanger Dowex 50WX8 incorporated. The defined porosity size of the hydrogels was adjusted by the addition of a solid water-soluble porogen (NaCl). Neurotransmitters (NTs) or their analogs were bound to the ion exchanger. Five groups of modified hydrogels populated with SPC-01 cells were established [no NTs (DWX0), L-dopamine (DWX1), serotonin (DWX2), tryptamine (DWX3) and carbachol (DWX4)]. The groups DWX0-4 were tested in vitro, and the two most promising candidates, together with DWX 0 as a control, were tested in an in vivo model of rat spinal cord hemisection.

To investigate the effect of NTs released from the gels, a real time measurement proliferation assay was used. Gel samples 3x3x3 mm in size were added to E-plates 3 hours after seeding with 10 000 SPC-01 cells. Cell growth during 200h after seeding was monitored using an xCELLigence RTCA Instrument (Roche). A significant increase in proliferation in the presence of DWX4 (by 25%) and DWX3 (by 25-30%) was observed when compared to the DWX0 group, whereas DWX1 and 2 displayed the opposite effect.

The growth and distribution of cells in the gel were analyzed immunocytochemically after 2, 7, 14, 21 and 28 days in culture. Within the 28 day period, SPC-01 cells populated the whole volume of the hydrogel samples. The analysis of gel samples showed that the DWX3 and DWX4 hydrogels were superior in terms of the relative number of attached cells throughout most of the in vitro part of the experiment. After 28 days of in vitro hydrogel-SPC-01 cell cocultivation, 2x2x2mm pieces of DWX0 (control), DWX2 (serotonin was previously described as a differentiation supportive factor) and DWX4 (showed the ability to support cell proliferation and attachment) cell-polymer constructs as well as gels only were implanted into rat spinal cord hemisection cavities (level Th9). Twenty-eight days post-implantation, the animals were sacrificed, and the spinal cords were removed, stained and immunohistochemically analyzed using the Wizzard program (Axiovision, Zeiss). All hydrogel materials nicely adhered to the spinal cord tissue with either no or minor cavity formation. The SPC-01 cells survived very well in the hydrogel implants, and some of them migrated into the surrounding spinal tissue in both caudal and cranial directions as far as a few millimeters from the lesion site. All hydrogels (with or without cells) supported the ingrowth of endogenous axons (NF160) and blood vessels (RECA) inside the implant.

In conclusion, the in vitro study showed that the DWX4 hydrogels showed the best ability to support SPC-01 cell growth and attachment and, together with DWX4 hydrogels, created a supportive environment in vivo that was permissive for the ingrowth of endogenous tissue elements such as axons and blood vessels. The impact of NTs on in vitro and in vivo SPC-01 cell differentiation will be further analyzed.

Poster Board Number:210**NOTCH 3 SIGNALING GATES CELL CYCLE ENTRY AND LIMITS NEURAL STEM CELL AMPLIFICATION IN THE ZEBRAFISH ADULT PALLIUM**

Alunni, Alessandro¹, Krecsmarik, Monika¹, Bosco, Adriana¹, Galant, Sonya¹, Pan, Luyuan², Moens, Cecilia³, Bally-Cuif, Laure¹

¹CNRS, Gif sur Yvette, France, ²Division of Basic Science, Seattle, WA, USA, ³Division of Basic Sciences, Seattle, WA, USA

Maintaining the homeostasis of germinal zones in adult organs is a fundamental but mechanistically poorly understood process. The control of stem cell activation, a key parameter of homeostasis, remains unclear. Adult neurogenesis in the mammalian brain remains a spatially and quantitatively limited phenomenon confined to two subregions of the telencephalon. Unlike in rodents, the zebrafish adult brain displays widespread neurogenesis, with sixteen characterized constitutively active neurogenic regions, including the ventricular zone of the telencephalon I.

In the adult zebrafish telencephalon, two types of progenitors have been identified along the ventricular zone (VZ): progenitors expressing glial markers (radial glia, RG) and intermediate neuronal precursors 2,3. At any given time point, the majority (90%) of RG is quiescent, while 10% is proliferating. Using lentiviral transduction, we demonstrated that RG are capable at the single cell level of both self-renewing and generating different cell types; hence exhibit bona fide NSC properties in vivo⁴. We also showed that RG predominantly undergo symmetric gliogenic divisions, amplifying the NSC pool, which may account for its long-lasting maintenance. Notch signaling limits neural progenitor proliferation in the adult zebrafish pallium.

Long-term analyses, as well as neurosphere and reconstitution assays, indicate that mouse adult germinal zones are progressively depleted in NSCs upon Notch inactivation⁵⁻⁷.

Combining pharmacological and genetic manipulations, we demonstrate here that long-term Notch invalidation primarily induces NSC amplification through their activation from quiescence and increased occurrence of symmetric divisions. Expression analyses, morpholino-mediated invalidation and the generation of a notch3-null mutant directly implicate Notch3 in these effects. In contrast, abrogation of notch1b function results in the generation of neurons at the expense of the activated NSC state. Together, our results support a differential involvement of Notch receptors along the successive steps of NSC recruitment. They implicate Notch3 at the top of this hierarchy to gate NSC activation and amplification, protecting the homeostasis of adult NSC reservoirs under physiological conditions.

Poster Board Number:211**NEW STRATEGIES FOR THE GENERATION OF MOUSE AND HUMAN DOPAMINERGIC NEURONS**

Caiazzo, Massimiliano¹, Dell'anno, Maria Teresa¹, Theka, Ilda², Dvoretzkova, Elena³, Leo, Damiana⁴, Lazarevic, Dejan⁵, Curreli, Sebastiano⁴, Taverna, Stefano⁴, Gainetdinov, Raul R.⁴, Gustinich, Stefano⁶, Dityatev, Alexander⁴, Broccoli, Vania¹

¹Division of Neuroscience, Ospedale San Raffaele, Milano, Italy, ²Gene Regulation, Stem Cells and Cancer, Center for Genomic Regulation, Barcelona, Spain,

³Department of Neuroscience and Physiology, New York University Langone Medical Center, New York, NY, USA, ⁴Neuroscience and Brain Technologies Department, Italian Institute of Technology (IIT), Genova, Italy, ⁵Center for Translational Genomics and Bioinformatics, Ospedale San Raffaele, Milano, Italy, ⁶Neurobiology sector, International School for Advanced Studies (SISSA), Trieste, Italy

Lineage-specific transcription factors (TFs), which drive cellular identity during embryogenesis, have been shown to convert cell fate when expressed ectopically in heterologous cells. Remarkably, developmental fate molecular switchers are sufficient to force neuronal conversion in differentiated somatic cells of other germinal layers. We showed that it is possible to use key TFs in order to generate a specific neuronal subtype by direct reprogramming of fibroblasts. Indeed, we found a minimal set of three dopaminergic (DA) TFs (ASCL1, NURR1

and LMX1A) able to elicit direct conversion of mouse and human fibroblasts into DA neuronal cells. Molecular and transcriptome studies showed that mouse induced DA neuronal (iDAN) cells recapitulate gene expression of their brain homolog cells while lacking expression of other monoaminergic neuronal subtype markers. Mouse iDAN cells also showed spontaneous electrical activity organized in regular spikes consistent with the pacemaker activity featured by brain DA neurons. Moreover we proved that transplanted mouse iDAN cells are able to ameliorate the symptoms of a rat model of Parkinson's disease. Subsequently we focused our studies on the improvement of human iDAN cell generation. To this aim we screened new culture conditions and molecular factors able to increase the number and the differentiation of human iDAN cells. Our results showed that combining the hypoxic culture condition and the use of miRNA-9/9* and -124 is possible to increase the maturation of human iDAN cells and also to obtain an efficiency of conversion close to that of mouse iDAN cells. Finally we showed that ASCL1, NURR1 and LMX1A are also able to speed up and simplify the generation of functional DA neurons from human induced pluripotent stem (hiPS) cells. In conclusion we proved that forcing the expression of key DA TFs is amenable to generate reprogrammed DA neurons from both from mouse and human fibroblasts or hiPS cells. These findings might have significant implications in studies of neural development, in vitro modeling of neurodegenerative diseases and cell replacement therapies.

Poster Board Number:212**ABNORMALITIES OF HIPPOCAMPAL NEUROGENESIS AND PROLIFERATION IN THE R6/1 MOUSE MODEL OF HUNTINGTON'S DISEASE**

Pakpoor, Jina, Begeti, Faye, Barker, Roger
Cambridge University, Cambridge, United Kingdom

Introduction: Huntington's disease (HD) is an autosomal dominant neurodegenerative condition characterized by movement disorders, psychiatric disturbance, and cognitive decline. Previous work has demonstrated a reduction in adult hippocampal neurogenesis in transgenic mouse models, known to be important in hippocampal-dependent cognition. It is widely accepted that neurogenesis occurs in two distinct areas of the adult mammalian brain; the subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus.

Objectives: To examine the abnormalities of hippocampal neurogenesis and proliferation in the R6/1 mouse model of HD and explore the role of stem/neural precursor cells in abnormalities of adult hippocampal neurogenesis.

Methods: In 16 week old R6/1 mouse four commonly used markers were used to stain the dentate gyrus. The stained cells were subsequently quantified. Mouse anti-rat Nestin and rabbit anti-sox2 were used to identify stem/neural precursor cells; rabbit anti-Ki67 was used to identify proliferating cells; and goat anti-DCX to identify neurogenesis.

Results: Proliferating cells and immature neurons were significantly reduced in the dentate gyrus DG of R6/1 mice, compared to WT littermates [Ki67: $t = 6.23$, $P < 0.001$, DCX: $t = 7.46$, $P < 0.001$]. The number of nestin+ processes transversing the DG was significantly increased in R6/1 mice [$t = -4.182$, $p = 0.002$], compared to WT. There were no differences between the number of Sox2+ cells in HD and WT mice.

Conclusions: This study shows that in addition to the well-described reduction in proliferation and immature neurons in the R6/1 HD model, there is a robust increase in nestin+ cells indicating that the deficit in adult hippocampal neurogenesis occurs at the neural stem/progenitor cell. Despite a large increase in nestin+ processes, the number of sox2+ cells was unchanged between genotypes, which could be explained by an increase in astrogliosis in the DG. It is unclear whether these quiescent cells could be somehow manipulated in order to restore the neurogenesis impairment in HD mouse models. However, given that the increase in nestin+ cells was consistently

observed in both cohorts of R6/1 mice examined in this study as well as in the more progressive R6/2 mouse model (Clelland et al. unpublished data), changes in this marker may be useful as a new outcome parameter to assess the efficacy of treatments aiming to restore the neurogenesis deficit in HD mice.

Poster Board Number:213

OSVZ RADIAL GLIA-LIKE STEM CELLS IN THE HUMAN MEDIAL GANGLIONIC EMINENCE

Nicholas, Cory R.¹, Wang, Xiaojun², Harwell, Corey C.³, Chen, Jiadong¹, Wang, Shaohui¹, Rubenstein, John L.R.⁴, Alvarez-Buylla, Arturo⁵, Kriegstein, Arnold R.¹

¹Department of Neurology, University of California, San Francisco, San Francisco, CA, USA, ²Institute of Biophysics, Chinese Academy of Sciences, Beijing, China,

³Department of Neurobiology, Harvard, Boston, MA, USA, ⁴Department of Psychiatry, University of California, San Francisco, San Francisco, CA, USA, ⁵Department of Neurological Surgery, University of California, San Francisco, San Francisco, CA, USA

Radial glia stem cells line the ventricular epithelium of the developing nervous system and generate neuronal and glial cell lineages. We and others have described an additional radial glia-like stem cell population in the outer sub-ventricular zone (OSVZ) of the mouse, ferret, and human neocortex that generates cortical excitatory neurons and may promote increased cortical size and complexity in humans. However, it is unknown whether the medial ganglionic eminence (MGE), the birthplace of most cortical inhibitory interneurons, contains diverse radial glia-like stem cells.

Here, we find that the human fetal MGE contains an abundant fraction of OSVZ radial glia-like (oRG) stem cells. Similar to cortical oRGs, oRGs of the MGE are unipolar and undergo mitotic somal translocation, a characteristic behavior whereby the cell body quickly translocates toward its primary fiber and divides. Unlike cortical oRGs, however, MGE oRGs are not radially aligned with long basal fibers but instead display short fibers with basal, apical, or tangential orientation that often retract during division. Using live time-lapse imaging and clonal analysis in slice cultures, we found that MGE oRG stem cells can divide asymmetrically to self-renew and differentiate, generating both unipolar translocating oRGs and bipolar non-translocating intermediate progenitor daughter cells. Furthermore, we detected many oRG daughters that expressed ASCL1, indicative of differentiation toward a neuronal lineage. We also found that dissociated cultures of human fetal MGE, and pluripotent stem cell-derived MGE-like cells, maintained oRG-like stem and daughter cell properties in vitro. Thus, human MGE progenitors include a non-epithelial radial glia-like stem cell type with unique orientation and behavior that is located outside of the ventricular zone and lacks attachment to the apical adhesion belt or the basal lamina. Human MGE oRGs likely provide increased production of inhibitory interneurons to complement the elevated number of excitatory neurons produced in the human neocortex.

Poster Board Number:214

CD200 AND HLA-A,B,C ENABLE THE ISOLATION OF NEURONS FROM NEURAL INDUCTION CULTURES OF HUMAN EMBRYONIC STEM CELLS

Corselli, Mirko, Vidal, Jason G., Emre, Nil, Martin, Jody, Carson, Christian T. *BD Biosciences, San Diego, CA, USA*

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. 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. Cell sorting 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.

Poster Board Number:215

BIOLOGICAL PROPERTIES OF THE NEURAL CREST-DERIVED MULTIPOTENT STEM CELLS FROM A BULGE REGION OF ADULT HAIR FOLLICLE AT CLONAL LEVEL

Vasyliov, Roman¹, Rodnichenko, Angela¹, Zubov, Dmitriy¹, Shestakova, Tetyana², Litvinova, Larisa³, Rybachuk, Oksana⁴, Labunets, Irina¹, Novikova, Svetlana¹, Butenko, Gennadiy¹

¹Cell and Tissue Technologies, State Institute of Genetic and Regenerative Medicine, NAMS of Ukraine, Kiev, Ukraine, ²R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kiev, Ukraine, ³Innovation Park, Laboratory of Immunology and Cell Biotechnology, Immanuel Kant Baltic Federal University, Kaliningrad, Russian Federation, ⁴Bogomoletz Institute of Physiology, NAS of Ukraine, Kiev, Ukraine

Objective: The bulge region (BR) of a hair follicle (HF) contains the neural crest-derived multipotent stem cells (NC-MSCs) [Sieber-Blum M., 2004]. BR HF NC-MSCs obtained by explant method have a potential to differentiate into the melanocytes, neurons, Swann cells, smooth muscle cells and chondrocytes. A characteristic feature of the NC-MSCs from other adult tissues is their capacity to spherogenesis. The aim of this study was to determine the ability of the BR HF NC-MSCs for self-renewal, directed multilineage differentiation and spherogenesis at clonal level.

Material and methods: NC-MSCs were isolated by explant method of M. Sieber-Blum from the BR HF of whisker pad from adult FVB mice. Cells were cultured on collagen type I in DMEM:F12 medium with 10% FCS, 2 ng/ml bFGF, 1% B27 and 1% MEM vitamins under 5% O₂ and 5% CO₂. The capacity of cells to clonal growth was assessed by seeding of 100 cells (P1) per 100 mm Petri dish. For experiments the colonies containing more than 100 cells (500-1000 cells avg) have been selected and subcultured with cloning cylinders. The capacity of cells to self-renewal was determined via reseeded of a clonal colony and culturing at clonal density. Differentiation into adipocytes and osteoblasts was carried out via conventional protocols. For neuronal differentiation the NC-MSCs were cultured in Neural Base Medium P with 10% mouse brain extract, 1% B27, 2% NeuroMix, 2 μM retinoic acid, 5 ng/ml bFGF and 20 ng/ml EGF on poly-L-lysine. For Schwann cells differentiation the above medium was supplemented with 100 nM isoproterenol. For serum-free growth the NC-MSCc were cultured in DMEM:F12 with 2% B27, 40 ng/ml bFGF and 40 ng/ml EGF. Differentiation was assessed via cytochemical staining with Alizarin Red (for osteoblasts), Oil Red (for adipocytes), immunofluorescent staining with anti-βIII-tubulin mAbs (for neurons, 1:500), anti-S-100 mAbs (1:500) and secondary FITC-conjugated antibodies (1:2000). The animals were treated in accordance with "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" [Strasbourg, 1986].

Results and conclusions: In CFU-test a capacity of the fibroblast-like cells from explants of BR HF to growth at clonal density was shown. A colony forming efficiency was 72.58±12.35%. It was marked that fibroblast-like cells at P1 were heterogeneous by their proliferative potential at clonal density. Depending on cell number and morphology,

all clonal colonies can be ranked by three types: colony type I consisted of 8-10 large cells with distinct morphological features of differentiation (13.86±2.74% of the total number of colonies); colony type II - 30-70 cells of different morphology (31.68±8.35%), colony type III - over 100 (500 cells avg) of small cells with absent morphological signs of differentiation (54.46±7.58%). When subcloning of a colony type III (n=15), the formation of new colonies of all types has been observed: type I - 28.25±2.78%; type II - 40.9±7.04%; type III - 31.88±4.36% of a total number of newly formed colonies. CFU-test performing revealed a hierarchical structure of the fibroblast-like cell population from BR HF. Clonal cultures derived from the colonies type III possess a capacity to spherogenesis in a serum-free culture and directed differentiation into adipocytes, osteoblasts, neurons and Schwann cells. This study revealed the NC-MSCs from BR HF have ability to self-renewal, spherogenesis and multilineage differentiation at clonal level.

Poster Board Number:216

HUMAN PLURIPOTENT STEM CELL-DERIVED RADIAL GLIAL-LIKE CELLS WITH STABLE REGIONAL IDENTITIES

Ladewig, Julia¹, Ostermann, Laura¹, Müller, Franz-Josef², Koch, Philipp¹, Brüstle, Oliver¹

¹University of Bonn, Bonn, Germany, ²University of Kiel, 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 exhibits 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 differentiation 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.

Poster Board Number:217

DIFFERENTIAL GENOMIC IMPRINTING OF IGF2 REGULATES ITS PARACRINE VERSUS AUTOCRINE FUNCTION IN ADULT NEUROGENESIS

Radford, Elisabeth J.¹, Kleine, Ira¹, Domingo-Muelas, Ana², Ramme A, Anja², Gray, Dionne¹, Ferguson-Smith, Anne C.¹, Ferrón, Sacri R.²

¹Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom, ²Biología Celular, Universidad de Valencia, Burjassot (Valencia), Spain

The imprinted Insulin-like growth factor 2 (Igf2) gene is not imprinted in the choroid plexus and leptomeninges of the brain. Using an Igf2-reporter mutant mouse, we address the relationship between Igf2 imprinting and adult neurogenesis in the subventricular zone (SVZ)

and hippocampus in vivo. Paracrine IGF2 functioning as a CSF-derived neurogenic factor requires biallelic expression with reduced activation of the stem cell pool in the SVZ and impaired olfactory bulb (OB) neurogenesis in mutants. In contrast, in the hippocampus Igf2 is imprinted, and is an autocrine factor that is paternally expressed in the NSCs themselves. Reduction in the stem cell population is observed in the dentate gyrus (DG) but only when the Igf2 mutation is paternally transmitted. Our data also show that biallelic Igf2 expression may control the dosage of paracrine IGF2 which is taken up by the NSCs in the SVZ via activation of insulin receptor (IR) and/or insulin growth factor 1 receptor (IGF1R). In the DG the action of imprinted autocrine IGF2-signalling likely involves a different mechanism. Our findings emphasize the importance of selective imprinting in the control of adult neurogenesis, and indicate that a regulatory decision to imprint or not may be an important functional mechanism of dosage control.

Poster Board Number:218

LOSS OF SMN CAUSES NEURONAL DEFECTS FOLLOWING IN VITRO AND IN VIVO DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS

Chang, Wei-Fang¹, Liu, Ji-Long², Sung, Li-Ying¹

¹Institute of Biotechnology, Taipei, Taiwan, ²Department of Physiology, Anatomy and Genetics, MRC Functional Genomics Unit, Oxford, United Kingdom

Survival motor neuron (SMN) protein is the determining factor for spinal muscular atrophy (SMA), the most common genetic cause for infant mortality. Although several degenerative effects have been observed in SMA patients, it remains elusive whether and how developmental defects are associated with SMA. Here we establish a model in mouse embryonic stem cells (ESCs) to study the in vitro and in vivo differentiation ability by shRNA knockdown. Following knockdown of mouse Smn1 in ESCs, the in vitro neuronal differentiation was performed by adding retinoic acid (RA) and sonic hedgehog (Shh). We found that the number of neuritis displayed significantly lower in the Smn1 depleted ESCs compared to the control group. The early neuronal marker PAX6 and mature neuronal marker MAP2 were down-regulated. Furthermore, teratoma assay showed that neuronal markers such as betaIII-tubulin and neuron stem cell marker Nestin were down-regulated, indicating the ability of neuronal differentiation was affected in vitro and in vivo. Collectively, our work demonstrated that loss of Smn1 causes developmental defects at early differentiation stage, which might provide a useful platform to get mechanistic insights into SMA.

Poster Board Number:219

TIMP-1 MODULATES CHEMOTAXIS OF HUMAN NEURAL STEM CELLS VIA CD63 AND INTEGRIN SIGNALING

Kim, Jung Mi¹, Cho, Soo Young², Lee, Young Seek³, Lee, Myung Ae¹

¹Brain Disease Research Center, Ajou University School of Medicine, Suwon, Republic of Korea, ²Mammalian Genetics Unit, Medical Research Council Harwell, Harwell, United Kingdom, ³Division of Molecular Life Science, Hanyang University, Ansan, Republic of Korea

We recently reported that human neural stem cells (hNSCs) have interesting characteristics to migrate toward an intracranial glioma. However, the molecules and mechanisms responsible for tumor tropism are unclear. In this study, we identified a novel chemotactic molecule, tissue inhibitor of metalloproteinase-1 (TIMP-1), secreted from human brain tumor tissues using microarray and proteomics analysis. We demonstrated that TIMP-1 significantly enhanced hNSC adhesion and migration in a cell culture system. These effects were critically dependent on CD63 signaling, as short hairpin RNA-mediated ablation of CD63 expression attenuates the response. Knockdown of CD63 by RNA interference resulted in decreased hNSC spreading and migration, whereas control HBI.F3 dramatically increases the number of FAs and cell matrix adhesions for cell migration with TIMP-1 treatment. In addition, TIMP-1 binding to CD63 activated

b1-integrin-mediated signaling via Akt and FAK phosphorylation, subsequently leading to distribution pattern changes of vinculin and F-actin. Furthermore, inactivation of integrin b1 by blocking antibody or inhibition of phosphoinositide 3-kinase (PI3K) signaling impaired the migration of NSCs toward TIMP-1. Collectively, our results underline TIMP-1 as a novel and effective key regulator of CD63 and b1 integrin-mediated signaling controlling hNSC adhesion and migration.

Poster Board Number:220

ABETA42/40 RATIOS IN HUMAN NEURONAL CULTURES ARE RESISTANT TO PHARMACOLOGICALLY RELEVANT CONCENTRATIONS OF GAMMA-SECRETASE MODULATORS

Koch, Philipp, Mertens, Jerome, Stüber, Kathrin, Wunderlich, Patrick, Ladewig, Julia, Walter, Jochen, Brüstle, Oliver
University of Bonn, Bonn, Germany

Altered production, accumulation and aggregation of amyloid beta peptides ($A\beta$) in the brain are hallmarks of sporadic and familial Alzheimer's disease (AD). As increasing evidence suggests that elevated fractions of the highly amyloidogenic $A\beta$ 42 variant are causative in AD pathogenesis, γ -secretase modulators (GSM) that selectively lower $A\beta$ 42 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 $A\beta$ and that familial AD patient-specific iPSC-derived neurons exhibit an elevated $A\beta$ 42/40 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 $A\beta$ 42/40 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.

Poster Board Number:221

NEURAL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS IN 3D CULTURES

Bozza, Angela¹, Coates, Emily E.², Incitti, Tania¹, Messina, Andrea¹, De Filippis, Roberta¹, Fisher, John P.², Casarosa, Simona¹
¹Centre for Integrative Biology (CIBIO), University of Trento, Trento, Italy, ²Fischell Department of Bioengineering, University of Maryland, Maryland, MD, USA

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 the adhesion protein fibronectin (fn) or 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 are still alive at the end of the protocol. Quantitative RT (qRT)-PCR analyses showed that cells grown in alginate and alginate-HA increase differentiation toward neural lineages with respect to the 2D control and to fn group, with higher expression levels of the neural markers BIII-tubulin and NCAM. Immunocytochemistry analyses confirmed these results both in alginate and alginate-HA experimental groups, further showing terminal differentiation of these neurons, as seen by the expression of synaptic markers. Our data show that alginate, alone or modified, could be a suitable biomaterial to increase in vitro differentiation of pluripotent cells toward neural fates.

Poster Board Number:222

DERIVATION OF TRACEABLE AND INTEGRATION-COMPETENT PHOTORECEPTORS FROM MOUSE EMBRYONIC STEM CELLS

Decembrini, Sarah¹, Koch, Ute², Moulin, Alexandre³, Radtke, Freddy², Arsenijevic, Yvan¹

¹UNIL Université de Lausanne, Lausanne, Switzerland, ²École Polytechnique Fédérale de Lausanne, Institut Suisse de Recherche Expérimentale sur le Cancer, Lausanne, Switzerland, ³Eye Pathology Laboratory, Jules-Gonin Eye Hospital, 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 immature and mature photoreceptors, and assessed the extent to which this protocol recapitulates photoreceptor development in vitro. Using a 3D-culture system, we generated retinas in vitro able to produce GFP positive photoreceptors between days 12 and 14 of culture, reaching the peak of birth between days 18 and 20 of culture. Similarly, the alignment of photoreceptors increased over time up to day 25. Transplantation of ES cell-derived photoreceptors sorted at different days of culture is feasible but showed a different degree of integration as assessed by the number of grafted cells. The peak of integration was reached transplanting photoreceptors at day 25 of culture. Integrated cells showed the typical morphology of mature photoreceptors with the production of external segments, spherule synapses and the expression of rod-specific proteins such as Rhodopsin, Pde6B and GNAT1. While massive and aggressive pigmented tumour formation was detected in retinas grafted with unsorted cells just 3 weeks after injection, no tumour formation was found after transplantation of sorted GFP-positive cells. We conclude that 3D-culture system coupled with ES cells provides a safe and renewable source of photoreceptors displaying a transplantation competence comparable to photoreceptor cells derived from age-matched retinas.

Poster Board Number:223**CHARACTERIZATION AND DIFFERENTIATION OF HIPS CELLS FROM MULTIPLE SCLEROSIS PATIENTS****Sampaio, Gabriela¹**, Laterza, Cecilia¹, Peitz, Michael², Matino, Gianvito¹¹Neuroimmunology, San Raffaele Scientific Institute, Milano, Italy, ²Institute of Reconstructive Neurobiology, University of Bonn, Bonn, Germany

Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system in which focal lymphocytic infiltration cause damage to myelin and axons. The cause of multiple sclerosis remain unknown, however some evidences suggest that MS patients have a genetic predisposition that could be triggered by environmental factors. The in vitro study of affected cell types, could represent a precious tool to better understand the mechanisms involved in the development of MS and to develop more efficacious treatments. However, obtaining biopsies from the patient's neural tissue is very difficult and the material obtained cannot be maintained in culture and studied.

A new source of stem cells, recently obtained by genetic reprogramming of somatic cells – the so-called induced pluripotent stem cells (iPSCs) – may represent the appropriate tool to overcome these problems. The method first described by Yamanka consists into the direct reprogramming of somatic cells, i.e. fibroblasts, through induced expression of four transcription factors (OCT3/4, SOX2, KLF4 and c-MYC).

Indeed iPSCs can be derived directly from patient own cells and are in vitro expandable, almost indefinitely, and differentiated into all the three germ layers cell types.

iPSCs have been already used to study many neurodegenerative disease in vitro, for disease modeling, drug screening and as a possible tool for regenerative medicine. The possibility to differentiate these cells allows studying the diverse cell types involved in the disease from the same source, avoiding invasive procedures.

Our aim is 1) to obtain iPSC lines from MS patients and healthy donors, 2) to characterize the iPSC lines obtained from MS patients and healthy donors, 3) to differentiate these cells into neurons and 4) to compare the features of healthy neurons and neurons derived from MS patients.

We first derived iPSC lines from fibroblasts obtained by three different patients with divers forms of MS (Benign, Relapsing Remitting, Primary Progressive) and form two healthy controls sex and age matched with patients. iPSC lines were obtained by fibroblast infection with non integrating sendai virus vectors. We characterized iPSC lines by RT-PCR and immunofluorescence for pluripotency related markers (e.g. Oct 3/4, Nanog, Sox2, SSEA3, SSEA4, TRA1-81, TRA1-60). Moreover, we assessed the ability to differentiate into the three germ layers by embryoid body formation also characterized by RT PCR. Finally, we induced neuronal differentiation using a dual SMAD inhibition protocol and we characterized the neuronal populations obtained at different time points. Preliminary data arising from immunostaining experiments and RT-PCR-based analysis of the expression of neural-specific RNA species suggests that iPSCs are able to differentiate into neural progenitors and neurons.

Current experiments are aimed at deeper characterize the neuronal populations obtained from iPSCs and to compare healthy vs. MS derived neurons.

Poster Board Number:224**TLR5 STIMULATES NEURONAL DIFFERENTIATION VIA IL-6 INDUCTION IN MOUSE EMBRYONIC STEM CELL****Kim, Won-Jae¹**, Choi, Seong Ho², Kim, Seon-Mi³, Kim, Sun-Hun⁴, Jung, Ji-Yeon²¹Chonnam National University, Gwangju, Republic of Korea, ²Oral Physiology, Chonnam National University, Gwangju, Republic of Korea, ³Pediatric Dentistry, Chonnam National University, Gwangju, Republic of Korea, ⁴Oral Anatomy, National University, Gwangju, Republic of Korea

Recent reports have showed that several TLR family members were expressed in mouse embryonic/ adult stem cells, suggesting that these may play a role in stem cell activities. To elucidate the possibility

that TLRs play a functional role in neural differentiation from mouse embryonic stem cell (mESC), we studied the expressional pattern of TLRs and these's signal mechanism under all-trans retinoic acid (ATRA) -treated differentiation media. Toll like receptor 5 (TLR5) was significantly upregulated and NeuroD1, DCX and NeuN, neuronal makers, were upregulated in mESC exposed with ATRA containing media. To verify a role of TLR5 on neural differentiation from mESC, we transfected TLR5-overexpression vector or TLR5-shRNA in mESC. Over-expression of TLR5 upregulated NeuroD1 and beta III tubulin expression in mESC exposed with neuronal differentiation media compared to control. However, down-regulation of TLR5 reduced both NeuroD1 and beta III tubulin expression and dendrite outgrowth relative to control group. In addition, over-expression of TLR5 upregulated IL-6 expression in mESC, whereas down-regulation of TLR5 decreased it's expression. Taken together, our data show that TLR5 enhances neuronal differentiation from mESC through regulating IL-6 expression.

Poster Board Number:226**NM23 REVERTS HUMAN STEM CELLS TO THE NAÏVE STATE, INCREASES EFFICIENCY OF IPS GENERATION AND PRODUCES CELLS THAT DIFFERENTIATE IN A MANNER SUPERIOR TO THAT OF CELLS CULTURED IN FGF****Bamdad, Cynthia C.**, Smagghe, Benoit, Stewart, Andrew, Carter, Mark G., Shelton, Laura

Minerva Biotechnologies, Waltham, MA, USA

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 a 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. In addition to culturing existing stem cells, we used NM23 in a minimal feeder-free, defined system to generate human iPS cells. Substituting NM23 for FGF decreased the time required for induction of pluripotency, increased efficiency by more than 100-fold and eliminated the need for c-Myc.

When allowed to passively differentiate, NM23-cultured stem cells showed no germline preference. However, they differentiated in a coordinated way with as much as 90% of cells in a local environment differentiating down the same germline. When cells were directed to differentiate into a specific cell type, pre-culture in NM23 resulted in as much as a 40-fold increase in the efficiency of differentiation over the standard method.

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. Whether NM23 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 they likely 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.

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".

Poster Board Number:227

ESTRADIOL PROMOTES PROLIFERATION AND ODONTOGENIC DIFFERENTIATION OF HUMAN DENTAL PULP CELLS

Jung, Ji-Yeon¹, Kim, Seon-Mi², Kim, Sun-Hun³, Kim, Won-Jae¹

¹Oral Physiology, ¹Chonnam National University, Gwangju, Republic of Korea, Democratic People's Republic of, ²Pediatric Dentistry, ¹Chonnam National University, Gwangju, Republic of Korea, ³Oral Anatomy, ¹Chonnam National University, Gwangju, Republic of Korea

Objective: Estradiol is known to be an endogenous estrogen could positively influence the proliferation or differentiation of neural stem/progenitor cells. However, the biological effects of estradiol in human dental pulp cells (HDPCs) have not been well studied. This study investigated the effects of estradiol on the proliferation and odontoblast/osteoblast-like differentiation in HDPCs.

Methods: For differentiation condition, cells were cultured in differentiation inductive medium including 100µM/L ascorbic acid and 10 mM/L β-glycerophosphate. Cell viability, proliferation, levels of messenger RNA for differentiation-related genes, alkaline phosphate (ALP) activity, and mineralization were assessed in HDPCs treated with various concentrations of estradiol.

Results: Estradiol stimulated cell viability in a dose-dependent manner. BrdU positive cells and expression of proliferating cell nuclear antigen (PCNA) were increased in estradiol-treated HDPCs, compared with control. However, estradiol increased the ALP activity, and enhanced formation of mineralized nodule. Moreover, estradiol upregulated odonto/osteoblastic markers including ALP, bone sialoprotein (BSP), dentin matrix protein-1 (DMP-1) and dentin sialophosphoprotein (DSPP), compared with untreated control. In addition, estradiol increased phosphorylation of Erk, p38 and JNK in differentiation condition.

Conclusion: These findings suggest that estradiol promotes proliferation and odontoblastic differentiation of HDPCs.

Poster Board Number:228

PROTEIN KINASE INHIBITOR SU6668 ATTENUATES POSITIVE REGULATION OF GLI PROTEINS IN MULTIPOTENT PROGENITOR CELLS

Piirsoo, Alla¹, Piirsoo, Marko², Kasak, Lagle¹, Neuman, Toomas¹

¹Protobios, Tallinn, Estonia, ²Tallinn University of Technology, Tallinn, Estonia

Observations that Glioma-associated transcription factors GLI1 and GLI2 (GLI1/2), executors of the Sonic Hedgehog (SHH) pathway and targets of Transforming Growth Factor β (TGF-β) signalling axis, are involved in numerous developmental and pathological processes unveil them as attractive pharmaceutical targets. Unc-51-like serine/threonine kinase ULK3 has been suggested to play kinase activity dependent and independent roles in the control of GLI proteins in the context of the SHH signalling pathway. Here, human adipose tissue derived stromal cells (ASCs) are investigated as experimental model possessing both TGF-β and SHH signalling axes functional. We demonstrate that cultured ASCs differentiate towards osteoblasts in response to

SHH. Also, we identify SU6668 ((Z)-5-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-propanoic acid) as an inhibitor of ULK3 catalytic activity and a compound targeting GLI proteins in an ULK3-dependent manner in cell-based assays. SU6668 prevents de novo expression of GLI1/2 proteins and antagonizes the GLI-dependent activation of the gene expression programs induced by either SHH or TGF-β. Our data suggest SU6668 as an efficient inhibitor allowing manipulating the GLI-dependent transcriptional outcome.

Poster Board Number:229

IDENTIFICATION OF MIRNAS THAT MODULATE THE FUNCTION OF BMP4 AND NODAL/ACTIVIN PATHWAYS IN PLURIPOTENT STEM CELLS

Parisi, Silvia¹, Battista, Marica², Musto, Anna¹, Navarra, Angelica¹, Russo, Tommaso³

¹Department of Molecular Medicine and Medical Biotechnology, University of Naples, Naples, Italy, ²Ceinge, Naples, Italy, ³Department of Molecular Medicine and Medical Biotechnology, University of Naples, Naples, Italy

miRNAs have a crucial role in pluripotency and differentiation of Embryonic Stem cells (ESCs). There is an increasing body of evidence that miRNAs may act by integrating the extracellular signals with the complex transcriptional network that regulate ESC fate. Two branches of TGFβ signalling pathway, i.e. BMP4 and Nodal/Activin, are key regulators of ESC state and they act by controlling the main steps of ESC differentiation as well the induction of pluripotency through the reprogramming. Many papers indicate that the role of these signals is usually opposite and perfectly balanced so that a mis-regulation of one of these pathways has relevant consequences on the other one. We have recently demonstrated that the fine balance between these two pathways is regulated by the miR-125a at least in the first phases of ESC. This regulation goes through an efficient auto-regulatory loop by which BMP4 controls the transcription of miR-125a that targets the BMP4 co-receptor; *Dies1*, and in turn this mechanism controls the proper response of ESCs to the BMP4 stimulus. Moreover, we have found that also the other miRNA of the same family, miR-125b, is able to target *Dies1* and to control the transition from ESCs into epiblast stem cells suggesting that these two miRNAs work cooperatively through the suppression of *Dies1*. This relationship is an elegant example of how multiple miRNAs can converge on a single pathway to promote a common outcome. Interestingly, while miR-125a is directly regulated by BMP4, miR-125b seems to be not regulated by TGFβ signalling. This observation suggests that the BMP4 signalling in the first steps of ESC differentiation undergoes different regulations that are dependent (miR-125a) or independent (miR-125b) by itself. This can be probably due the relevance that the balance between BMP4 and Nodal/Activin pathways have in the control of the transition from ESC to epiblast stage, thus indicating that ESCs modulate in different ways such important pathways. Our working hypothesis is that the same type of control of the cell fate is adopted in various critical steps of differentiation or induction of pluripotency. Starting from this idea we want to identify the miRNAs that are directly regulated by BMP4 and/or Nodal/Activin and how these miRNAs modulate ESC differentiation and reprogramming, through the balance of these two pathways. At this aim, we have already identified the miRNAs that are directly regulated by BMP4 at transcriptional level in the early phase of ESC differentiation. Once we have identified the miRNAs regulated by BMP4 and Activin, we will study the effects that modulation of these miRNAs may have on ESCs and on reprogramming. These results, along with target identification, will allow us to retrace the functional network driven by TGFβ in ESCs and during reprogramming and, moreover, to select single miRNAs or pools of them able to control the differentiation or to improve the efficiency of reprogramming.

Poster Board Number:230**C-MYC INDUCES A STEM-CELL LIKE PHENOTYPE IN MAMMARY EPITHELIAL CELLS THROUGH WNT PATHWAY REGULATION**

Poli, Vittoria¹, Stefania, Mazzolini¹, Luca, Fagnocchi¹, Hiroshi, Hatsuda¹, Alessandro, Cherubini¹, Riccardo, Serafini², Alessio, Zippo¹

¹Epigenetics Unit, National Institute of Molecular Genetics - INGM, Milan, Italy,

²Biotechnologie, Università Degli Studi Di Siena, Siena, Italy

A strong reduction in breast cancer death rate has been achieved through early cancer detection and new pharmacological treatments. Notwithstanding, critical improvements in knowledge of disease pathogenesis in term of biology and molecular mechanisms are still required. Cell lines are defined as bona fide stem-cell lines when are characterized by long-term self renewal and multipotency. The tumorigenicity is the gold-standard in order to define a stem-cell line as a bona fide cancer stem cell (CSC) line. In normal stem cells the self-renewal is tightly controlled by signals including Wnt, Hedgehog and Notch pathways. The improperly regulation of these pathways confers to normal cells stem-cell like characteristics leading to the arising of tumor-initiating cells. In this scenario, it seems that c-Myc, one of the major Wnt target genes, plays a pivotal role by activating the stem-cell like transcriptional program. Notably, the Wnt pathway, which is fundamental for the normal mammary development, has been retrieved de-regulated in breast cancers. Accordingly, c-Myc is frequently amplified in breast cancers.

Recently, it has been demonstrated that c-Myc over-expression leads to an hyper-activation of the Wnt pathway through the transcriptional repression of the Wnt antagonist DKK-1 and SFRP-1. This finally promotes the encourage-independent cellular growth of immortalized human mammary cells. We set out to determine whether c-Myc over-expression in immortalized human mammary epithelial cells (IMEC) may lead to the acquisition of cancer stem cell-like traits through the activation of the Wnt pathway. The sphere forming efficiency (SFE) assay, also known as mammospheres assay, is reported to be a powerful tool in order to identify cells bearing stem-cell-like features within a heterogeneous population. By applying this assay to IMEC cell line over-expressing or not c-Myc (IMEC c-Myc or IMEC wt) we measured the ability of a single cell to give rise to a clone in non-adherence condition. Only c-Myc over-expressing cells were able to form mammospheres starting from a single cell (M1 clones). Moreover, the generated M1 clones when challenged through subsequently sub-cloning (M2, M3 clones) where enriched in the stem cell-like content compared to the parental heterogeneous population of origin. By purifying single cells characterized by the Wnt pathway activation highlighted by a TCF reporter system, we observed a drastic enrichment in the M1 spheres forming efficiency. To assess if the observed sphere forming efficiency induced by c-Myc rely also on the Wnt pathway we checked the clonogenic efficiency by adding co-activators and inhibitors of the Wnt signaling pathway to the medium. Notably, co-activators such as Wnt3a and R-SPONDIN-1 were not sufficient to induce spheres formation in IMEC wt line, while the antagonist DKK-1 and SFRP-1 strongly reduced the spheres forming ability of c-Myc over-expressing cells. Accordingly, genome wide microarray analysis showed that the major inhibitors of the Wnt pathway, DKK-1 and SFRP-1, were down-regulated in IMEC c-Myc cell line compared to IMEC wt. Moreover, this transcriptional silencing was amplified in M1, M2 and M3 clones compared to their parental heterogeneous c-Myc over-expressing line.

All these data suggest a pivotal role of c-Myc in regulating the stemness traits exploiting, at least in part, the Wnt pathway.

Poster Board Number:231**EFFECT OF NOTCH PATHWAY INHIBITION ON DIFFERENTIATION POTENTIAL OF HUMAN PERICYTES**

Pierantozzi, Enrico, Curina, Carlo, Vezzani, Bianca, Badin, Margherita, Sorrentino, Vincenzo

Molecular and Developmental Medicine, University of Siena, Siena, Italy

Notch signaling is involved in the regulation of cell proliferation, cell-fate determination and differentiation of diverse embryonic and adult progenitor cells. In post-natal life Notch signaling regulation is closely related to both self-renewal and terminal differentiation of tissue stem cells although it may exerts opposite effects depending on the cell type. Mesenchymal Stem Cells (MSCs) are one of the most studied adult progenitor cells and represent a promising cell population to be used in tissue engineering or other cell-based therapies. It has been recently proposed that microvascular pericytes may represent the ancestor cell population of all MSCs throughout the body as they are able to differentiate toward cells of mesodermal lineages, including muscle and bone. However, it is not clear if pericytes from different tissues respond in the same way to specific differentiation stimuli. We analyzed the effect of Notch signaling inhibition by DAPT, a γ -secretase inhibitor, in hPCs isolated from skeletal (sk-hPCs) and smooth (sm-hPCs) muscle tissues. Both sk-hPCs (n=7) and sm-hPCs (n=7) expressed known hPCs markers as assessed by FACS, IF, RT-PCR and WB experiments. Interestingly, we found that only sk-hPCs were able to differentiate along myogenic and osteogenic lineages, while sm-hPCs were not. To investigate the involvement of Notch pathway in differentiation potential of hPCs, cells were treated with DAPT during proliferation and/or following induction of differentiation. We found that DAPT-treated cells from both tissues had a population doubling and growth rate significantly lower than control cells, although cell survival was not affected. In sk-hPCs, myogenic efficiency of DAPT-treated cells was three fold higher than control cells. In addition, early myogenic genes such as MyoD and Myf5, and skeletal muscle microRNAs such as mir1 and mir206, were significantly up-regulated in DAPT-treated sk-hPCs. Interestingly, also the expression of linc-MD1 was up-regulated in DAPT-treated cells. The increase of myogenic efficiency of sk-hPCs was however effective only when DAPT was added during both proliferative and differentiative stage. In contrast, osteogenic potential of sk-hPCs was abolished in DAPT-treated cells as indicated by both qualitative and quantitative analysis of mineral matrix deposition and Real-Time PCR of osteogenic gene expression. DAPT administration during the differentiation period was sufficient to suppress osteogenic potential of sk-hPCs, while DAPT treatment before induction of differentiation was not able to prevent the differentiation process. In contrast, DAPT treatment in sm-hPCs did not affect either myogenic or osteogenic differentiation. Taken together, these results indicate that only skeletal but not smooth muscle pericytes can differentiate in myogenic and osteogenic cells. Furthermore, in sk-hPCs, the inhibition of Notch signaling by DAPT stimulated myogenic differentiation, while osteogenic differentiation was prevented.

Poster Board Number:232**DIFFERENTIATION DYNAMICS AND TUMORIGENIC POTENTIALS OF MOUSE EMBRYONIC STEM AND EMBRYONIC TERATOCARCINOMA CELLS ARE GOVERNED BY DIFFERENT TGF β SIGNALING REGULATION**

Gordeeva, Olga

Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russian Federation

Pluripotent stem cells are able to proliferate indefinitely and differentiate in vitro into multiple somatic cell types. However, in vitro differentiation of the pluripotent stem cells is asynchronous and incomplete, and therefore, the residual undifferentiated cells can initiate teratoma development after transplantation into recipient tissues. This feature of the pluripotent stem cells is the major issue for the

development of safe pluripotent stem cell-based therapy. In order to understand mechanisms underlying differentiation of pluripotent stem cells, retinoid acid (RA) induced differentiation of mouse embryonic stem (ES) cells and their malignant counterparts teratocarcinoma stem (EC) cells were investigated. The growth and differentiation dynamics of ES R1 and EC F9 cells after RA exposure for 10 days were studied and then tumorigenic potentials of resulted cell populations were evaluated after their transplantation into immunodeficient nude mice. A significant decrease of growth rates and numbers of undifferentiated Oct4+ cells in both cell lines treated by RA for 10 days was found. However, after additional 3 days of RA withdrawal the number of Oct4+ cells in differentiating ES cell populations significantly increased as opposed to EC cells. The assessment of tumorigenic potential of differentiating cell populations showed that ES and EC cells RA treated for 5 days retained the ability to form teratomas and teratocarcinomas. However, after 10 days of RA treatment only ES cells formed teratomas whereas RA-treated EC cells were not capable to develop teratocarcinomas. In order to clarify molecular mechanisms of ES and EC cell differentiation we have investigated the expression of cell lineage marker genes and TGF β family factors. Gene expression analysis demonstrated marked differences in the expression patterns of TGF β family ligands in ES and EC cells. Therefore, ActivinA and BMP4 were applied together with RA to induce differentiation of ES cells. At the end of the experiments a significant decrease of the residual Oct4+ cells was found in both cases as compared with control ES cells RA exposed. Thus, we suppose that TGF β family ligands and their target genes differently regulate lineage determination upon induced differentiation of ES and EC cells. Therefore, multichoice cell fate decisions in course of pluripotent stem cell differentiation require more effort to be limited.

Poster Board Number:233

THE ACTIVATION OF WNT/ β -CATENIN PATHWAY IN SALIVARY GLAND STEM/PROGENITOR CELLS

Maimets, Martti¹, Pringle, Sarah Ann¹, Huch, Meritxel², Vries, Robert², Clevers, Hans², van Os, Ronald³, Coppes, Rob¹

¹Department of Cell Biology, University Medical Centre Groningen, Groningen, Netherlands, ²Hubrecht Institute for Developmental Biology and Stem Cell Research, Utrecht, Netherlands, ³Department of Biology of Aging, European Research Institute for the Biology of Aging (ERIBA), Groningen, Netherlands

Radiation-induced damage to normal tissues may result in organ dysfunction and cause a reduction in the patient's post-treatment quality of life. Radiotherapy of head and neck cancer patients, which often involves co-irradiation of the salivary glands, induces hyposalivation with concomitant symptoms such as oral dryness, dental caries, oral infections, and difficulties with speech and food mastication. Regenerative therapy of the salivary gland is a promising therapeutic approach to prevent those consequences. However, little is known about the interplay between key signal transduction pathways and their molecular regulators of stem/progenitor cell activity and regenerative processes in the salivary gland. For example, Wnt/ β -catenin and Notch signaling regulate the function of many adult stem cell populations but their role in salivary gland development and regeneration is unknown. Previously, upon inflicting damage to the adult salivary gland we detected an upregulation of Wnt target gene Lgr5 during the regeneration process. Therefore, the aim of this project is to investigate molecular pathways involved in salivary gland tissue maintenance and regeneration.

Cells from mouse submandibular glands were isolated and cultured for 3 days into salispheres. Salispheres were collected, disrupted into single-cell solution and plated into three-dimensional culture system. The medium was supplemented with Wnt3a and R-spondin-1, Wnt/ β -catenin signaling pathway inducing factors. Under these conditions a significant increase in salispheres formation and expansion was observed as early as 7 days after treatment. These cultures have been expanded more than 3 months by weekly passaging. Without

Wnt3a and R-spondin-1, the cultures deteriorated within three to four passages. Similar expansion was observed using cells derived from human parotid and submandibular glands.

After growing salisphere-derived cells in three-dimensional culture system with expansion medium for 8 days, a formation of organoids resembling the embryonic salivary gland was observed. To distinguish them from organoids associated with our previous work showing differentiation into mature salivary gland cell lineages, the term 'neoglands' was used.

The salispheres were mainly formed by cells with high proliferation capacity, as shown by Ki67 staining, and contained all the cell types present on the salivary gland: ductal cells (CK7 and CK18 positive cells) as well as acinar cells (Aquaporin-5 and SMGC positive cells).

In conclusion, by supplementing our defined in vitro culture with factors inducing the Wnt/ β -catenin pathway, we are able to create long-lived self-organizing salivary gland structures in the absence of non-epithelial cellular niche. By passaging these structures we are able to expand the salivary gland stem/progenitor cell pool, which will be an invaluable tool for salivary gland stem cell and cancer research.

Poster Board Number:234

WIDESPREAD CHROMATIN REMODELING DURING HUMAN ENDOTHELIAL CELL REPROGRAMMING

Vassina, Ekaterina M.¹, Sokolov, Alexey S.², Zhenilo, Svetlana V.², Bogomazova, Alexandra N.¹, Mazur, Alexander², Prokhorchuk, Egor B.², Kiselev, Sergey L.¹, Lagarkova, Maria A.¹

¹Stem Cell Laboratory, Vavilov Institute of General Genetics, Russian Academy of Science, Moscow, Russian Federation, ²Centre 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. We also performed functional enrichment analysis for each set of gene transcripts and their expression status. 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. This approach revealed the differential organization of H3K27me3-enriched regions in H3K27me3-only transcripts compare to 'bivalent domains'. Silencing of most of somatic genes, except HUVEC-specific transcription factors, were accompanied 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 and were associated with metabolic processes. Our integrated data analysis will help to reveal the complex machinery that defines cell identity.

Poster Board Number:235**A MECHANICAL CHECKPOINT CONTROLS MULTICELLULAR GROWTH THROUGH YAP AND TAZ REGULATION BY ACTIN CAPPING AND SEVERING FACTORS**

Aragona, Mariaceleste, Panciera, Tito, Manfrin, Andrea, Giulitti, Stefano, Michielin, Federica, Elvassore, Nicola, Dupont, Sirio, Piccolo, Stefano
University of Padova, Padova, Italy

Key cellular decisions, such as proliferation or growth arrest, typically occur at spatially-defined locations within tissues. Loss of this spatial control is a hallmark of many diseases, including cancer. Yet, how these patterns are established is incompletely understood. We here report that physical and architectural features of a multicellular sheet inform cells about their proliferative capacity through mechanical regulation of YAP and TAZ, known mediators of Hippo signaling and organ growth. YAP/TAZ activity is confined to cells exposed to mechanical stresses, such as stretching, location at edges/curvatures contouring an epithelial sheet, or stiffness of the surrounding extracellular matrix. We identify the F-actin capping/severing proteins Cofilin, CapZ and Gelsolin as essential gatekeepers that limit YAP/TAZ activity in cells experiencing low mechanical stresses, including contact inhibition of proliferation. We propose that mechanical forces are overarching regulators of YAP/TAZ in multicellular contexts, setting responsiveness to Hippo, WNT and GPCR signaling.

Poster Board Number:236**FORCED EXPRESSION OF SOX17 CONVERTS MOUSE EMBRYONIC STEM CELLS (MECS) INTO FUNCTIONAL EXTRAEMBRYONIC ENDODERM STEM (XEN) CELLS**

McDonald, Angela¹, Biechele, Steffen¹, Stanford, William L.², Rossant, Janet¹
¹*Developmental and Stem Cell Biology, The Hospital for Sick Children, Toronto, ON, Canada*, ²*Regenerative Medicine Program, Sprout Centre for Stem Cell Research, Ottawa, ON, Canada*

In addition to the embryo proper, the mammalian conceptus generates two extraembryonic tissues, the trophoblast 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. 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. To determine XEN conversion efficiency, single-cell Sox17-mediated conversion was performed and found to be highly efficient, with over 90% of cells converting by day 30. Transgene silencing is observed following Sox17 over-expression and these cells can be cultured independent of transgene expression for greater than 30 passages. Upon injection

into host blastocysts, stable Sox17-XEN cells integrate and proliferate into the parietal endoderm of E6.5, E7.5 and E8.5 embryos. To identify dynamic regulatory networks involved in Sox17-mediated XEN conversion, time series RNA-sequencing was performed, revealing distinct stages of gene expression during conversion. Our findings suggest that Sox17 expression disengages the mESC regulatory network while assembling the XEN regulatory network. Further bioinformatic analysis will identify downstream nodes in the XEN regulatory network.

Poster Board Number:237**DEVELOPMENT OF A NEW PLATFORM FOR ANTICANCER DRUG SCREENING**

Tręda, Cezary, Stoczyńska-Fidelus, Ewelina, Walczak, Maciej, Winięcka-Klimek, Marta, Jankiewicz, Marcin, Hufas-Bigoszewska, Krystyna, Piaskowski, Sylwester, Rieske, Piotr, **Grzela, Dawid P.**
Celther Polska, Lodz, Poland

Many of anti-EGFR small molecule drugs cannot be tested in vitro without difficulties. This is particularly true for chemical compounds designed against mutated form of the receptor described in literature as EGFRvIII. Tumour cells spontaneously showing this alteration in vivo have failed to be cultured under in vitro conditions. For this reason cell lines showing EGFRvIII expression have to be genetically engineered. Until now, we have developed a new platform for anti-EGFRvIII compound testing. We have created EGFRvIII expression vectors driving the gene of interest from strong heterologous promoter. In addition, we made EGFRvIII fusion constructs with fluorescent protein tags, which allows tracing protein turnover and subcellular localisation in real time. These genetic tools were then used for generation of several cell lines displaying stable expression of EGFRvIII.

Poster Board Number:238**HTAF4-TAFH CO-ACTIVATOR DOMAIN OF TAF4 ACTIVELY AFFECTS CELL DIFFERENTIATION PROCESS IN HUMAN MESENCHYMAL STEM CELLS AND NORMAL HUMAN NEURAL PROGENITORS CELLS**

Kazantseva, Jekaterina, Neuman, Toomas, Palm, Kaia
Cellin Technologies, Tallinn, Estonia

Transcription factor TAF4 is a subunit of TFIID complex and a part of the general transcriptional preinitiation complex. Recent data shows that activity of general transcriptional machinery is not static or ubiquitous, but can be regulated in spatio-temporally manner during organism development and cell differentiation. Thus, among other conventional TFIID components, expression of co-activator TAF4 is heterogeneous across development and non-canonical TAF complexes actively participate in diverse cell differentiation processes. We found that expression of TAF4 alternative splice variants is diverse in different human organs, tissues and various regions of human brain, suggesting a role of alternative splicing in the control of TAF4 activity during organism development. Interesting, the most affected by alternative splicing part of TAF4 is a co-activator TAFH domain. Majority of alternative splice variants of TAF4 have deletion or extra insertion of hTAF4-TAFH, changing its helical structure and respective functional binding with transcription activators. For functional study of hTAF4-TAFH on cell differentiation, we used hTAF4-TAFH RNAi analysis of human adipose-derived MSCs and normal human neural progenitor cells with further differentiation stimulation. We found that TAF4 isoforms with structurally modified co-activator hTAF4-TAFH domain promote chondrogenic differentiation of hMSCs and accelerate neurogenesis in neural progenitor cells. Moreover, intactness of hTAF4-TAFH is a necessary condition for successful adipogenic and osteogenic turnover of hMSCs. Furthermore, our results demonstrate new notion about involvement of TAF4 in p53 and non-canonical WNT pathways signaling upon cells differentiation.

Poster Board Number:239

MICROSCALE TECHNOLOGIES FOR REGULATING HUMAN STEM CELL DIFFERENTIATION

Cimetta, Elisa, Sirabella, Dario, Tandon, Nina, Yeager, Keith, De Berardinis, Elio, Vunjak-Novakovic, Gordana
Columbia University, New York, NY, USA

During development and regeneration, tissues emerge from coordinated sequences of stem cell renewal, specialization and assembly that are orchestrated by cascades of regulatory factors. This complex in vivo milieu is poorly replicable in vitro, while being necessary for biological research and generating progenitor cells. We are just starting to fully realize the importance of replicating the entire context of cell microenvironment - the other cells, three-dimensional (3D) matrix, and cascades of molecular and physical signals. Bioengineered environments that combine tissue-specific transport and signaling are critical to study development, regeneration and disease under settings predictive of human conditions. Microscale technologies offer potential for conducting more sophisticated experiments at biologically relevant scales and with real-time insights into cellular responses. We thus developed microbio-reactors coupling application of fast dynamic changes of environmental signals with versatile, high-throughput operations and imaging compatibility. Our base device comprises an array of microwells housing 3D cell constructs exposed to stable concentration gradients generated by an integrated microfluidic platform. Mathematical modeling of flow and mass transport predicts the gradients shape and the kinetics of concentration changes. A single microbio-reactor yields up to 120 data points, corresponding to 15 replicates of a gradient with 8 concentration levels. This system is very versatile, allowing for parameter modifications in a range of biological studies. We here list two examples: (i) Embryoid Bodies (EBs) obtained from human embryonic and induced pluripotent stem cells (hESC, hiPSC) were exposed to concentration gradients of Wnt3a, Activin A, BMP4 and their inhibitors, to investigate early-stage fate specification and mesodermal lineage commitment. We were able to evaluate the initiation of mesodermal induction by correlating gene expression profiles to concentration gradients of mesoderm-inducing morphogens. (ii) Xenopus embryos were exposed to gradients of depolarizing agents, and the effects of changing membrane potentials on cell development were studied. We also developed a new bioreactor platform that addresses a long-standing need for culture systems combining: (i) provision of precisely controlled 3D environments resembling the in vivo ones, (ii) application of multiple regulatory factors (biochemical, physical, mechanical), (iii) modular designs for high-throughput and combinatorial studies, and (iv) live imaging compatibility for real-time insight. The proposed system is based on a common platform providing a set of basic functions interfaced with on-line imaging, environmental control, and data acquisition. The focus is on generating functional myocardial tissue containing physiologically high density of viable cells (10^8 cells/cm³). The engineered tissues could then be implanted in the damaged heart or used as tissue mimics for drug screening and as disease models thus allowing more in depth understanding of cardiac disease development and reaction to specific drugs and stimulations. We propose that microbio-reactor systems, providing radical advance over existing technologies, can form a basis for predictable models of development and disease.

Poster Board Number:241

COPPER TRANSPORTER 1 (CTR1) REGULATES GERM LAYER SPECIFICATION

Brigden, Kurt W.¹, Main, Heather², Fraser, Stuart T.³
¹Discipline of Physiology and Bosch Institute, University of Sydney, Camperdown, Australia, ²School of Physics, University of Sydney, Camperdown, Australia, ³Discipline of Physiology, Discipline of Anatomy & Histology and Bosch Institute, University of Sydney, Camperdown, Australia

Copper is an essential trace element, serving as a cofactor for many important enzymes and proteins. Copper uptake occurs through the high-affinity copper transporter, Ctr1. Ctr1 null-mutant mice fail to gastrulate leading to embryonic lethality. This inability of Ctr1^{-/-} mice to form mesoderm has been phenocopied during in vitro differentiation of Ctr1^{-/-} embryonic stem cells (ES). However, the role of Ctr1 in development of ectoderm and endoderm has yet to be explored. To assess in vitro ectoderm generation, Ctr1^{+/+} and Ctr1^{-/-} ES cells were cultured as a monolayer in N2B27 medium to promote neuroectodermal differentiation. Ctr1^{+/+} ES cells formed rosette structures, containing nestin⁺ neural stem cells and NeuN⁺ neurons. Absence of Ctr1 results in reduced neural differentiation, evident through the delayed generation of mature NeuN⁺ neuronal cells. Ctr1^{-/-} ES cells gave rise to surface ectoderm at an earlier time point than Ctr1^{+/+} ES cells, resulting in larger and more frequent ectodermal colonies. In contrast to mesoderm and ectoderm development, Ctr1^{-/-} and Ctr1^{+/+} ES cells gave rise to equal numbers of CXCR4⁺ c-kit⁺ endodermal progenitors. In conclusion, Ctr1^{-/-} ES cells fail to generate mesoderm however can give rise to both endoderm and ectoderm. Under neuroectodermal growth conditions, Ctr1^{-/-} ES show a lineage bias towards surface ectoderm production.

Poster Board Number:242

FOCAL ADHESION KINASE REGULATES HUMAN EMBRYONIC STEM CELL SURVIVAL

Vitillo, Loriana¹, Whiting, Paul², Kimber, Susan¹
¹Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom, ²Neusentis, Pfizer, Cambridge, United Kingdom

Undifferentiated human embryonic stem cells (hESCs) grow on the extracellular matrix (ECM) substrate fibronectin (FN) in defined feeder-free conditions. The ECM plays a pivotal role in the maintenance of the hESCs pluripotent niche but the contribution to survival remains to be elucidated. Understanding the mechanism of survival is particularly crucial in hESCs because of their known high sensitivity to cell death, affecting cell culture and ultimately translation of research to the clinic. hESCs bind to FN mainly via $\alpha 5 \beta 1$ -integrin, known to be upstream of important survival cascade in other cell types. However, it is not understood if and how the FN/integrin binding supports those molecular pathways in the context of pluripotent hESCs. The aim of this work was to elucidate the survival cascade downstream of the FN/integrin interaction in hESCs. Initially, we showed that when hESCs were cultured on a non-integrin activating substrate they initiated an apoptotic response, shown by a 7-fold increase in the apoptotic marker Annexin V. We then selectively blocked $\beta 1$ -integrin with antibody and shown a dose-dependent apoptotic response. Integrin activation is generally transduced to the cells via a complex adhesion of scaffold and kinase proteins, among which the focal adhesion kinase (FAK) plays a key role. Indeed, blocking $\beta 1$ -integrin resulted in dephosphorylation of endogenous FAK in hESCs. When FAK kinase activity was directly inhibited (with small inhibitor), hESCs responded by detaching from FN and activating caspase-3, leading to an increase in apoptosis. Furthermore, flow cytometry analysis showed that the population of hESCs that undergo apoptosis still retained the pluripotency-associated marker NANOG. FAK is a convergent point with growth factor signalling and the PI3K/AKT pathway, with a well-reported role in the maintenance of hESCs. Consistently, FN activated both AKT and its target MDM2 protein levels, while pAKT was reduced after $\beta 1$ -integrin blocking and FAK

inhibition. Cell imaging showed that the ubiquitin ligase MDM2, which regulates p53 degradation, displayed reduced nuclear localization after treatments, opening the possibility for a change in the p53 balance in hESC. In fact, p53 protein increases after FAK inhibition corresponding also to caspases activation. Further investigation will explore if FAK-dependent pathways are implicated in the maintenance of the hESCs in non-canonical ways. In conclusion, the work contributed to define a survival role of the ECM substrate FN based on activation of β 1-integrin and FAK, resulting in the activation of AKT signalling to prevent cell death.

Poster Board Number:244

THE FLATWORM MACROSTOMUM LIGNANO IS A POWERFUL MODEL FOR STEM CELL RESEARCH

Grudniewska, Magda K., Wudarski, Jakub J., Mouton, Stijn, Berezikov, Eugene

Stem Cell Regulation and Mechanisms of Regeneration, European Research Institute for the Biology of Ageing, Groningen, Netherlands

The environment of stem cells is essential for the regulation of their function. As it is complex to simulate the natural environment in vitro, it is important to use different model systems to learn more about the fundamental aspects of in vivo stem cell biology. One of the emerging models is the free-living flatworm *Macrostomum lignano*. This species is convenient for in vivo research of how stem cells are regulated to replace missing, damaged, and aged tissues, as it has a pluripotent population of stem cells, called neoblasts.

In order to further advance *M.lignano* as a new model, its genome and transcriptome draft assemblies were recently generated, and methods for making transgenic animals are being developed. Moreover, additional RNA-Seq libraries were and will be made from: irradiated adults devoid of proliferating cells, worms in different developmental stages, aged worms, and aged regenerated worms. These libraries can be used for different purposes.

To screen for novel, evolutionary conserved, stem cell genes, we compared libraries of control adults, irradiated adults, and juveniles. This resulted in a gene candidate list, which was studied in detail using in situ hybridization, RNA interference and antibody labeling of proliferating cells. We identified previously known as well as novel genes with crucial functions for stem cell functionality during homeostasis and regeneration.

To screen which pathways are important for stem cell ageing and rejuvenation, we will compare the libraries of aged non-regenerated and aged regenerated (and thus potentially rejuvenated) worms. This poster will present *Macrostomum lignano* as a new model organism for in vivo stem cell research.

Poster Board Number:245

GENERATION OF MULTIPOTENT STEM CELLS

Walczak, Maciej, Winięcka-Klimek, Marta, Grzela, Dawid P., Skrzypczyk, Aniela, Stoczyńska-Fidelus, Ewelina, Hulas-Bigoszewska, Krystyna, Rieske, Piotr, Piaskowski, Sylwester
Celther Polska, Lodz, Poland

The recent discovery of nuclear reprogramming to generate ES-like induced pluripotent stem cells (iPSC) from isolated somatic cells, which share the properties but not ethical challenges of embryonic stem cells, offers new promising models for cellular therapies. The major concern with clinical application of iPSC is however their propensity to form tumours. In contrary, multipotent stem cells appear to be less dangerous for prospective treatments, since they are not the source of teratomas.

We have developed new method of converting somatic cells, where de-differentiation is performed without reversing to pluripotency state. By using transcription factor Sox2 with combination of additional proteins (included in order to change reprogramming efficiency), we obtained iNS cells (induced neural stem cells), characterized by neural

stem cells morphology, expression of NSC markers: GFAP and Nestin. Those cells in appropriate conditions can be differentiated into glial and neuronal cells.

Poster Board Number:246

RETROVIRUS INSERTION IN IPSCS IDENTIFIES GENES FACILITATING SOMATIC REPROGRAMMING

Nowrouzi, Ali¹, Aoi, Takashi², Herbst, Friederike¹, Deichmann, Annette¹, Arens, Anne¹, Kara, Nergis¹, Zimmermann, Philipp K.¹, Abel, Ulrich¹, Utikal, Jochen³, Glimm, Hanno¹, Schmidt, Manfred¹, Yamanaka, Shinya⁴, von Kalle, Christof

¹*Translational Oncology, NCT/DKFZ Heidelberg, Heidelberg, Germany,* ²*Department of iPS cell Applications, Kobe University, Kobe, Japan,* ³*Skin Cancer Unit, DKFZ Heidelberg, Heidelberg, Germany,* ⁴*Center for iPSC Research and Application, Kyoto University, Kyoto, Japan*

Transcription factor-induced reprogramming of induced pluripotent cells (iPSCs) de-stabilizes the differentiated state of somatic cells via intermediate cell populations. The identity and molecular functions of genes that promote reprogramming are highly relevant for understanding the mechanistic roadblocks to this reprogramming process. Generally, transferring the reprogramming factors by vectors that integrate stably into the genome is more efficient than non-integrating vectors, which suggests that vector-induced gene activation enhances reprogramming. By dissecting the complete insertional inventory of 12 mouse and 24 human derived iPSC, we show that vector insertions in iPSCs are selected intensely and non-randomly in proximity to genes expressed in embryonic stem cells and iPSCs. These insertions show significant ($p < 0,001$) and non-random enrichment in proximity to chromatin marks defining the transcriptional regulatory circuitry of embryonic stem cells (ESC) and genes regulating embryonic development. On several occasions, iPSC insertions even occurred into both alleles of the same gene. We found that following systematic selection and ectopic expression of these mechanistically-relevant genes tagged by retroviruses cellular reprogramming into iPSCs is significantly facilitated. Sequential stages of iPSC generation were enhanced more than 10-fold. Genes that facilitate reprogramming include chromatin modifiers as well as regulators of innate immunity and RNA Polymerase III activity. These findings explain the higher frequency of iPSC derivation with integrating vector systems, and predict heterogeneity among such iPSC clones. The capacity of such genes to enhance reprogramming indicates their biological role in overcoming molecular barriers to de-differentiation. Comprehensive and multi-stage insertional mutagenesis screens with retroviral- or transposon-based vectors enable a further functional dissection of the molecular mechanisms that enhance reprogramming and pluripotency. Functional analysis of these genes indicates that stochastic and deterministic roadblocks accompanying reprogramming can be identified and overcome in the search for more efficient and controlled iPSC derivation.

Poster Board Number:247

INHIBITION OF SIRT6 IN THE INTERMEDIATE STATE OF REPROGRAMMING FROM MOUSE EMBRYONIC FIBROBLAST (MEF) INCREASED INDUCED PLURIPOTENT STEM CELLS (IPSCS) FORMATION

Peng, Qian, Lee, Yinlau, Chen, Chunhung, Ng, Hungyu, Yeung, Shubiu
Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong, Hong Kong

SIRT6 (Sirtuin6), which belongs to histone deacetylase family, was initially implicated in longevity and its over-expression could extend the lifespan of male mice. Unlike SIRT1 which is also an anti-aging gene in the same family, SIRT6 is highly substrate-specific and regulates glucose metabolism through deacetylating H3K9Ac, as demonstrated by the fact that SIRT6-deficient cells had an increased glucose uptake and glycolysis level with reduced mitochondrial respiration. Previous studies in our laboratory have found that SIRT1 promoted iPSCs

formation by deacetylating p53 and regulating its downstream target gene, p21. The result in this study showed no significant changes in Sirt6 mRNA level in mouse ESCs and iPSCs when they underwent differentiation process through embryoid body formation. However, an increased Sirt6 mRNA was found during the maturation phase of reprogramming. Even though the level started to reduce from stabilization phase and was further decreased when those iPSCs were serially passaged, it was still higher than that of mouse ESCs, indicating an inhibitory role of SIRT6 in iPSCs formation. Therefore, SIRT6 was knocked down by siRNA treatment during reprogramming. The result showed that the blockage of SIRT6 in the maturation phase could promote the iPSCs formation, demonstrated by increased iPSCs colony number and Nanog mRNA level in the treated iPSCs. However, no such effect was observed in the iPSCs treated with SIRT6 siRNA on the initiation phase. The manipulation of SIRT6 did not affect the expression of p53 and p21 level but led to increased mRNA level of Glut (glucose transporter) 3, which is a glucose transporter with much more higher affinity for glucose than Glut1 and Glut2 and regulates the initiation steps of glycolysis during nuclear reprogramming. Indeed, dose dependent effect of glucose (0, 5, 25, 50, 100 mM) on reprogramming efficiency was found with the highest colony number in 5 mM and lowest in 0 and 100 mM glucose treated group. However, even though Glut1 was reduced along with increased glucose level, the mRNA level of Sirt6 and Glut3 in iPSC colonies was not affected by different glucose concentrations. Previous report showed that stimulation of glycolysis increased reprogramming efficiency. The results in this study demonstrated that the active inhibition of SIRT6 in the maturation phase of reprogramming led to the up-regulation of glycolytic genes-Glut3, which may increase the glucose flux into the cells and thus promote iPSCs induction. The data suggested that high level of SIRT6 in iPSCs may impair their pluripotency and further application for clinical therapy.

Poster Board Number:248

IN VITRO DEVELOPMENTAL POTENTIAL OF HUMAN EMBRYONIC STEM CELL LINES EVALUATED BY GENE EXPRESSION ANALYSIS OF A NEW SET OF DIFFERENTIATION MARKERS

Tesarova, Lenka, Koutna, Irena, Drdulova, Alena, Stejskal, Stanislav, Potesilova, Michaela, Simara, Pavel

CBIA, Masaryk University, Faculty of Informatics, Brno, Czech Republic

BACKGROUND: The ability to direct differentiation of human embryonic stem cells (hESCs) into specific cell types presents great promise for regenerative medicine. For example in vitro generated hematopoietic progenitors could provide an alternative to hematopoietic stem cells used for transplantations. However, the current limiting factor is the low efficiency of differentiation protocols and functional defects of derived blood progenitors. One of the issues to address these obstacles is to focus on the selection of original cells, as significant differences in the ability to differentiate into various germ layers and into hematopoietic cells were found among hESC lines. The aim of this study was to design the methodology to determine hESC differentiation potential with focus to hematopoietic differentiation and to evaluate this parameter in several hESC lines.

METHODS: Gene expression of twelve selected markers was monitored by RT-qPCR technology using hydrolysis probes from the Universal ProbeLibrary in multiplex assay with the Universal ProbeLibrary Reference Gene. hESC lines CCTL-12, CCTL-14, and HUES-1 were evaluated during their spontaneous differentiation induced by embryoid body formation using hanging drop method, while undifferentiated hESCs and cells from days three, seven, eleven, fifteen, and twenty-one of differentiation were analysed.

RESULTS: A set of differentiation markers was established representing three germ layers: mesoderm (Brachyury T, MIXL1), endoderm (AFP, SOX17) and ectoderm (NEFH, PAX6); hematopoietic and hematopoietic stem cells (KDR, PECAM1, PROM1, CD34); and pluripotent cells (NANOG, POU5F1) and including two housekeeping genes (G6PD, TBP). Expression pattern of these markers during hESC differentiation revealed their developmental potential. While CCTL-12 cell line demonstrated the potential to differentiate into all three germ layers, HUES-1 cell line showed skewed differentiation to endoderm layer and CCTL-14 cell line was characterised by preferential expression of ectoderm and mesoderm markers making this line the most suitable one for directed hematopoietic differentiation. Derivation of hematopoietic precursors defined by CD34 expression is inefficient during spontaneous differentiation of hESC, which was demonstrated by very low expression of this marker in all three developing cell lines in our study.

CONCLUSIONS: Optimal RT-qPCR conditions were determined for twelve newly designed assays enabling the sensitive gene expression analysis of selected differentiation markers. Using this set of markers developing pluripotent stem cells can be monitored under various conditions, enabling to evaluate the differentiation potential of these cells.

Poster Board Number:249

C-FLIP PROTECTS HUMAN PLURIPOTENT STEM CELLS FROM TRAIL INDUCED APOPTOSIS

HampI, Ales¹, Vinarsky, Vladimir¹, Krivanek, Jan¹, Sedlackova, Miroslava¹, Andera, Ladislav²

¹Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic, ²Laboratory of Cell Signaling and Apoptosis, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, 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, significantly less is known about expression and functionality of extrinsic apoptotic pathway. 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 proteosynthesis 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.

Poster Board Number:250**ALLELE-PREFERRED TARGETED CORRECTION OF CFTR GENE IN CYSTIC FIBROSIS INDUCED PLURIPOTENT STEM CELLS**

Davis, Brian R.¹, Bui, Jacquelin H.¹, Crane, Ana M.¹, Kramer, Philipp¹, Li, Xuan Shirley¹, Gonzalez-Garay, Manuel², Wang, Jianbin³, Paschon, David E.³, Guschin, Dmitry Y.³, Gregory, Philip D.³, Kotton, Darrell N.⁴, Holmes, Michael C.³

¹Center for Stem Cell & Regenerative Medicine, The University of Texas Health Science Center at Houston, Houston, TX, USA, ²Center for Molecular Imaging, The University of Texas Health Science Center at Houston, Houston, TX, USA, ³Sangamo BioSciences, Inc., Richmond, CA, USA, ⁴Boston University Pulmonary Center, Boston, MA, USA

Cellular transplantation of lung stem/progenitor cells represents a potential therapeutic approach for a variety of inherited monogenic diseases. Crucial to the success of such a therapeutic strategy is that the transplanted cells and their progeny are corrected for the disease-causing mutation and that the transplanted cells do not elicit an immune response in the recipient. In order to satisfy these criteria, we are pursuing a patient-specific approach in which, starting with skin or blood cells from patients with inherited lung disorders, autologous induced pluripotent stem cells (iPSCs) are first derived. Utilizing site-specific homology-directed repair, the disease-causing mutation is corrected in the endogenous, chromosomal DNA sequence. Finally, a directed differentiation approach is employed to obtain highly purified populations of the relevant stem/progenitor cells from the corrected iPSCs for purposes of transplantation.

We have employed this approach to generate corrected, autologous iPSCs for patients with Cystic Fibrosis (CF). Starting with skin fibroblasts of patients diagnosed with CF, we have derived and characterized iPSCs, confirming their pluripotency. We then utilized zinc finger nucleases (ZFNs), designed to target the endogenous CFTR gene, to mediate correction of the inherited genetic mutation in this locus via homology directed repair in these iPSCs. The corrected CF iPSCs retain normal karyotype, pluripotency, and a human ES-like expression profile. Correction was achieved for reprogramming transgene-containing as well as transgene-free CF iPSCs. The corrected CF iPSCs, when induced to differentiate in vitro, express the corrected CFTR gene. Importantly, we observed an exquisitely sensitive, homology-dependent preference for targeting one CFTR allele vs. the other. This allele-specific targeting offers the potential for preferential targeting of ZFN-mediated correction to dominant mutant alleles.

Poster Board Number:251**ALVEOLAR TYPE II CELLS TRANSPLANTATION DECREASE THE BLOOD-CIRCULATING FIBROCYTES AND THEIR MIGRATION TO THE LUNG IN THE PULMONARY FIBROSIS**

Guillamat-Prats, Raquel¹, Gay-Jordí, Gemma¹, Sánchez-López, Luis Ignacio¹, Sirenko, Valeria¹, Hernandez-Gonzalez, Fernanda¹, Bulbena, Oriol¹, Xaubet, Antoni², Serrano-Mollar, Anna¹

¹Patologia Experimental, IIBB-CSIC, Barcelona, Spain, ²Hospital Clínic de Barcelona, Barcelona, Spain

One of the most important features of IPF pathogenesis is epithelial cell injury and fibroblast proliferation. The original source of this aberrant fibroblast overpopulation might proceed from mesenchymal stem cells recruitment as circulating fibrocytes and its subsequent differentiation to fibroblasts/myofibroblasts. In a previous study our group demonstrated that intratracheal transplantation of isolated alveolar type II cells (ATII) can reverse the fibrotic process in a rat model of bleomycin induced lung fibrosis. The objective of this study was to determine whether ATII transplant was able to inhibit the mesenchymal stem cells recruitment as fibrocytes into the fibrotic lung. Lung fibrosis was induced by intratracheal instillation of bleomycin (3.5U/kg). The animals were transplanted with ATII (2.5x10⁶ cells/animal) 15 days after bleomycin instillation and were sacrificed 21 days

after the induction of fibrosis. The expression of CXCL12/CXCR4 axis was assessed by RT-PCR in Peripheral blood, lung tissue and isolated cells (macrophages and fibroblasts). Protein synthesis was analyzed by immunohistochemistry in tissue slices.

Fibrotic animals showed an increased expression of CXCR4 in peripheral blood and tissue. ATII transplantation was able to significantly reduce these increments. Furthermore, although fibrotic animals also showed increases in CXCL12 expression in pulmonary macrophages, these increases were inhibited in the fibrotic transplanted animals.

In conclusion ATII transplantation is able to inhibit the fibrocyte recruitment signals, reducing the fibrocyte infiltration to lung tissue and then stopping fibrosis.

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Poster Board Number:252**GENOMIC ENGINEERING OF THE CYSTIC FIBROSIS GENE IN PATIENT-DERIVED IPS CELLS**

Bachiller, Daniel¹, Camarasa, Mavi², Fleischer, Aarne², Asensio, Víctor², Gálvez, Víctor², Tokalov, Sergey²

¹Development and Regeneration, FISIB/CSIC, Bunyola, Spain, ²Development and Regeneration, FISIB, Bunyola, Spain

Cystic Fibrosis is a hereditary disease produced by the absence or malfunctioning of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. To date over 1,200 alterations in the DNA composition of the CFTR gene have been detected, although the deletion of the phenylalanine in position 508 (Δ F508) is responsible for more than 70% of the cases described in the European Population. CF is a degenerative disease, which can be considered as the main genetic cause of death in Caucasian children. Its first manifestations occur in early childhood, generally affecting the respiratory tract, and later extending to other organs.

The identification and isolation of the gene responsible for the disease raised great expectations of finding a treatment. However, such hopes have yet to be realized. Different attempts to develop effective gene therapy protocols have not provided satisfactory results.

We have opted instead for a genomic engineering approach, in which homologous recombination aided by gene editing nucleases has been used to eliminate the genetic defect of the gene.

The method was applied simultaneously in human and mouse cells, and includes the following steps: (I) Production of iPS cells from keratinocytes obtained from Δ F508 CF patients and Δ F508 mutant mice; (II) Correction of the mutation in the iPS cells with a combination of gene-editing nucleases and PiggyBac technology; (III) in vitro differentiation of repaired iPS cells up to a state in which the derived cell expresses CFTR; (IV) Functional analysis of the repaired CFTR protein.

Steps I through III have been completed and results will be provided at the meeting. Step IV is now under investigation.

Poster Board Number:253**NOVEL SINGLE CELL SLOW-FREEZING METHOD USING SERUM-FREE FREEZING MEDIUM FOR HUMAN PLURIPOTENT STEM CELL (HPSC) CULTURED IN FEEDER-FREE CONDITION**

Muramatsu, Marie, Nishishita, Naoki, Kawamata, Shin
Foundation for Biomedical Research and Innovation, KOBE, Japan

In contrast to vitrification, a slow-freezing is less labor intensive and has been studied elsewhere to cryopreserve human Embryonic Stem Cell (hESC) and human induced Pluripotent Stem Cell (hiPSC). However a recovery rate after slow-freezing is not comparable to cryopreservation by vitrification. This is partly because characteristic feature of hESC/iPSC, being primed or epistemic cell. They are maintained as colonies on feeder and passaged in cell clumps, not in single cells after dissociation from feeder and colony. Cryopreservation reagent shall

coat individual cells to exert protective effect during freezing and thawing on hESC/iPSC, so cell clump size and remaining cell surface molecules of hESC/iPSC after dissociation by dissociation buffers are critical to develop a slow freezing agent. Therefore, development of cryopreservation medium for hESC/iPSC shall always be couple with that of dissociation buffer.

In this report, we examine the recovery rate after thaw for hESC and iPSC maintained on vitronectin-coated dish in serum-free ReproFF (ReproCELL) and frozen in a combinatory use of various dissociation buffers (Accutage, TrypLE Select, Pronase and EDTA/PBS) and xeno-free cryopreservation mediums (CP-1 media, TC protector, Cell Baker 3 and CryoStem). The recovery rate of hESC/iPSC after single cell freezing and thawing was determined by number of viable cells after thaw against that before freezing. The recovery rate was the highest (over 36%) when hESC/iPSC were detached with TrypLE Select and cryopreserved with CP-1 media [final: 6% HES, 5% DMSO, 6% human albumin in saline]. It is not able that a simple formula CP-1 media has been used in clinic for the cryopreservation of cord blood cells in Japan for past 10 years. In thawing procedure, some 5000 single hES/iPS cells were seeded in one well of 96-well ultra-low attachment plate. Cells aggregated and form several hES/iPS cell clumps in 8 days of culture. Then hESC/iPSC clumps were transferred on vitronectin-coated 6 well-plate for expansion. Pluripotency-associated genes and molecule expression, cell proliferation rate, karyotype and three germ layers differentiation were determined in thawed hESC/iPSC after 5-10 passages. Single cells freezing and thawing of hESC/iPSC will eliminate technical bias of operators and enable us to examine the viability of hESC/iPSC before and after freeze/thaw processes. This novel feeder-free, serum-free and single cell preservation method would be suitable for cryopreservation of large amounts of hESC/iPSC and provide a robust, easy handling and economical cryopreservation method for hESC/iPSC bank for research and clinical use.

Poster Board Number:255

HOW TO BUILD AND MANAGE THE CELL MANUFACTURING UNITS FOR CLINICAL APPLICATION OF REGENERATIVE MEDICINAL PRODUCTS

Ueda, Toshio, Okura, Hanayuki, **Matsuyama, Akifumi**

Platform for Realization of Regenerative Medicine, Foundation for Biomedical Research and Innovation, Kobe, Japan

The cell manufacturing units (CMU) has become a facility indispensable for producing cell/tissue engineered medicinal products, which are made from various stem cells or ESCs/iPSCs. For clinical application, these cell-based medical products should be manufactured in CMU under the current Good Manufacturing Practice (cGMP) in which cells are cultured, proliferated, differentiated and filled into package. However, there is no standard for construction of CMUs to meet cGMP standard. Since the sterility and safety of cell products, which lack a sterilization process, are largely influenced by the producing process, constructions of CMUs are first issue. The CMUs hold a prominent position in the cGMP as facilities to prevent contamination by foreign matter, microbial contamination and cross-contamination from other products in all processes from importing of raw materials to shipment of final products. Fully equipped facilities should include layout of workrooms, layout/control of producing equipment and appropriate control of air control facilities in consideration of flow to keep air clean under manufacturing. Next, high hygienic control is required within the CMUs for the prevention of these events, because products containing foreign matter or contaminated by microorganisms must be treated as the defective products under regulation. Unnecessary animate beings for producing products should be eliminated from producing facilities. Especially insects and rodents are considered unhygienic animate beings, and sufficient care should be taken to avoid product contamination from their excreta and areas contacted by them. So, hygienic control should include establishment of disinfection method of facilities, confirmation method of disinfection effect/carrying-in method of raw materials and appropriate handling

method of infectious wastes. Third issue is training of workers/work management. The issue should also include establishment and compliance with the appropriate working method by performing training in hygienic control and manufacturing control according to a year-round training program. In the presentation, we proposed how to build and manage the CMUs for clinical application of regenerative medicinal products, focusing onto fully equipped facilities, hygienic control and training of workers/work management and to comply with them for meeting cGMP.

Poster Board Number:256

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

Suga, Mika¹, Kii, Hiroaki², Uozumi, Takayuki², Kiyota, Yasujiro², Furue, Miho Kusuda¹

¹Laboratory of Stem Cell Cultures, Department of Disease Bioresources, National Inst of Biomedical Innovation, Osaka, Japan, ²Nikon Corporation, Tokyo, 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.

Materials and Methods: Two human iPS cell lines, iPS-TIG114-4f1 and Tic (JCRB1437 and JCRB1331, respectively, JCRB Cell Bank, NIBIO, Osaka, Japan) were cultured and then stained with a cell-permeant SYTO24 green fluorescent nucleic acid stain (Invitrogen). Phase contrast and fluorescent images of the cells obtained intermittently in a cell incubator observation system, BioStation CT (Nikon Corporation) were analyzed by automated image analysis software CL-Quant (Nikon Corporation). Area of ES/iPS cell 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 to compare with the numbers of fluorescent nuclei.

Results: The fluorescent nucleus counting with images and the conventional cell counting by hemocytometer showed similar results. In the case of total cell numbers 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. The correlation curves were specific for each cell line.

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.

Poster Board Number:257**THE EUROPEAN HLA HOMOZYGOUS CORD BLOOD BANK REPROGRAMMING CONSORTIUM**

Wenet, Peter¹, Börger, Verena¹, Rebull, Paolo², Enczmann, Jürgen³, Madrigal, Alejandro⁴, Navarrete, Cristina⁵, Querol, Sergio⁶, Fibbe, Wim⁷, Devos, Timothy⁸, Greber, Boris⁹, Belmonte, Juan C.I.¹⁰, Santourlidis, Simeon¹¹, Adjaye, James¹²

¹Vivocell Biosolutions, Langenfeld, Germany, ²La Fondazione IRCCS Cord Blood Bank, Milano, Italy, ³Institute for Transplantation Diagnostics and Cell Therapeutics, Heinrich-Heine-University Hospital, Düsseldorf, Germany, ⁴Anthony Nolan Cord Blood Bank, London, United Kingdom, ⁵NHS Blood and Transplant Cord Blood Bank, London, United Kingdom, ⁶Banc de Sang I Teixits, Barcelona, Spain, ⁷LUMC, Leiden, Netherlands, ⁸UZ Cord Blood Bank, Leuven, Belgium, ⁹Max-Planck-Institute for Molecular Biomedicine, Münster, Germany, ¹⁰Center of Regenerative Medicine, Barcelona, Spain, ¹¹Epivios GmbH, Düsseldorf, Germany, ¹²Institute of Stem Cell Research and Regenerative Medicine, Düsseldorf, Germany

A consortium of European Cord Blood Banks pooling their HLA Homozygous allogeneic Cord blood units collected since up to 20 years has been initiated. At least 250 truly HLA homozygous CB units of distinct alleles shall be selected to reprogramming by advanced viral free technologies, mostly under GMP-conditions. A standardized informed consent form has been elaborated encompassing country- as well as European regulations. These HLA iPSC cell types of distinct ethnic backgrounds shall serve as selected per se isogenic cellular platform for their use in regenerative medicine.

Poster Board Number:258**DEVELOPMENT OF NOVEL TECHNOLOGIES FOR THE GENERATION OF CGMP-COMPLIANT INDUCED PLURIPOTENT STEM CELLS**

Kovarcik, Don Paul, Tian, Xinghui, Friedrich Ben-Nun, Inbar, Nie, Ying, Walsh, Patrick, Cadet, Jean, Burkall, Amy, Tran, Huan, Shah, Kevan, Ahmadian Baghbaderani, Behnam, Fellner, Thomas
LONZA, Walkersville, MD, USA

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.

Poster Board Number:259**SCALABLE PASSAGING OF HUMAN PLURIPOTENT STEM CELLS**

Kovarcik, Don Paul, Nie, Ying, Walsh, Patrick, Clarke, Diana, Rowley, Jon, Fellner, Thomas
LONZA, Walkersville, MD, USA

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™ I, 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 2x10⁵ hPSCs utilizing this passaging process exceeds 2x10¹² 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.

Poster Board Number:260**THE PLURIPOTENT STEM CELL SHARED RESOURCE FACILITY AT MOUNT SINAI**

Alexeeva, Vera¹, Rizvi, Hira¹, Cundiff, Paige¹, Lemischka, Ihor R.¹, Schaniel, Christoph², D'Souza, Sunita L.¹

¹Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY, USA, ²Department of Pharmacology and Systems Therapeutics, Icahn School of Medicine at Mount Sinai, New York, NY, USA

The maintenance of human embryonic stem cells and induced pluripotent stem cells (hESCs / iPSCs) and their differentiation into multiple lineages offers unprecedented opportunities to investigate and understand the earliest stages of human development. Like ESCs, iPSCs can self-renew thus providing us with a potentially unlimited source of cells. They can be genetically modified to define gene function and differentiated into multiple lineages. Furthermore, derivation of iPSC lines from individuals prone to various diseases also provides us with the opportunity to understand the etiologies of many complex syndromes. A dedicated pluripotent stem cell (PSC) shared resource facility (SRF), henceforth referred to as the 'Core', can aid principal investigators with pilot and/or continuing stem cell projects. The main objective of the pluripotent stem cell (PSC) shared resource facility (SRF) is to make the latest stem cell technology available to scientists. We currently use the Sendai virus and the mRNA/micro RNA methodology to reprogram blood and fibroblast cells respectively into iPSCs. The next step involves the differentiation of these cells into the diseased tissue-specific cells in order to recapitulate aspects of the disease. To facilitate the latter, we are generating various reporter systems using the latest Transcription Activator-Like Effector Nuclease (TALEN) and CRISPR technologies. These reporters will allow us to establish conditions necessary to improve the differentiation of specific

lineages and allow siRNA, micro RNA, small molecule/drug screens in novel cell populations. The second main objective of the Core is to quality control stem cell lines, reagents. Scientists are provided with quality controlled stem cell reagents at vastly discounted pricing made possible due to the establishment of three stem cell supply centers, bulk purchasing and an online ordering website. Quality control services include mycoplasma screening, karyotyping immunohistochemistry and gene expression for various stem cell markers. The third main objective of the Core is to continue to conduct classes to teach iPSC generation and hESC/iPSC cell differentiation into the lineage of choice. The goal of this education is to increase in the number of labs that are able to carry out stem cell research independently. Taken together, these services will have a three-fold benefit. Firstly, it will alleviate the quality control burden of individual scientists and allow them to concentrate on important scientific questions. Secondly, it will allow collaborative projects involving hESC / iPSC cell lines to be initiated with multiple laboratories by removing the prohibitive cost and providing the expertise required to establish and sustain this technology and lastly it will provide the Core with a source of revenue to meet its expenditures. Finally, with the assistance of Dr. Mahendra Rao, Director, National Institute of Health Center of Regenerative Medicine (NIH CRM), a group of 15 stem cell cores from all over the USA got together and set up the Stem Cell COREdinates group, www.coredinates.org, to further the idea of stem cell cores collaborating to promote the growth of stem cell science. Protocols shared within the group of CORES are being made available at both the COREdinates.org and Stembook.org websites.

Poster Board Number:261

REGULATORY ASPECT OF NON CLINICAL STUDIES FOR REGENERATIVE MEDICINE

Okura, Hanayuki, Senoo, Hachiro, Matsuyama, Akifumi

Platform for Realization of Regenerative Medicine, Foundation for Biomedical Research and Innovation, Kobe, Japan

Regenerative cell therapy is a discipline in youth, with only limited experience in clinical practice. Once manufacturing protocols of cells have been established in vitro studies, the manufactured cells were assigned as candidates for cell-based medicinal products. To judge whether the cell-based medicinal products manufactured according to the established protocols should be valuable for further development for clinical practice, non-clinical studies shall be conducted. In construction of a non-clinical study package for cell-based medicinal products, the non-clinical study package used for chemical compounds will help a lot. The requirements and contents of the non-clinical studies are classified into toxicity studies (general toxicity studies and special toxicity studies), pharmacological studies (primary pharmacodynamics studies and safety pharmacological studies), pharmacokinetic studies, formulation studies, and others. The non-clinical studies for the cell-based medicinal products are conducted to evaluate and qualify the efficacy and safety of the candidates. No wonder there is an argument about the selection of assessment parameters in toxicity, pharmacological and pharmacokinetics studies for cell-based medicinal product, because it is well-known that the cell-based medicinal products are different in properties from chemical compounds. Besides parameters used in these studies, the assessment issues may be an assumable minimum consensus, and others should be added on a case-by-case basis, depending on the cell manufacturing processes. In anticipation of the approval as the cell-based medicinal products, the possible requirements should be discussed here in the development process of such products from the standpoint of regulatory science, especially focusing on those in nonclinical studies.

Poster Board Number:262

RESEARCH FOR REGENERATIVE MEDICINE AND BIOETHICS

Okura, Hanayuki, Fukushi, Tamami, Matsuyama, Akifumi

Platform for Realization of Regenerative Medicine, Foundation for Biomedical Research and Innovation, Kobe, Japan

Ceaseless changes in research for regenerative medicine in response to new discoveries and technologies evoke discussions on how such innovations should be reconciled with our community as it exists now and as it will ideally exist in the future. To dissolve the issues, bioethics should be applied, but which what is required is not the discovery of new principles or foundations but instead the effort to identify important meanings and responses based on principles that we have already adopted. Therefore, bioethics in research for regenerative medicine should be applied in terms of the principles of respect for the person, beneficence, and justice. It can be said that the importance of consent as applied to medical research and of respect for the person as an expression of the underlying principle corresponding thereto, the importance of risk-benefit assessments as applied to research for regenerative medicine and of beneficence as an expression of the underlying principle corresponding thereto, and the importance of the choices made by test subjects as applied to the research and of justice as an expression of the underlying principle corresponding thereto have been reconfirmed in the history of bioethics. In this review, I would like to approach the framework of bioethics in the context of research for regenerative medicine from the perspectives of respect for the person, beneficence, and justice and explore the ideal form thereof from the perspective of bioethics.

Poster Board Number:263

TRANSLATIONAL GENOMICS AND STEM CELL RESEARCH: ETHICAL AND POLICY ISSUES

Al-Aqeel, Aida I.

Paediatrics, Prince Sultan Military Medical City, Riyadh, Saudi Arabia

We are at a time of unprecedented increase in knowledge of rapidly changing technology. Such biotechnology especially when it involves human subjects raises complex ethical, legal, social, regulatory and religious issues. One of these advances in translational genomics is stem cell research being seen as the best new hope in the search for cures to diseases. However, this research raises sensitive ethical, regulatory and religious arguments, which are balanced against possible great benefit of such research in regenerative medicine for patients suffering from so far incurable diseases. As the vast majority of new therapies that show promise in laboratories later prove unsafe and/or ineffective when tested in patients.

If the regulatory policies in each country are put forward for such research, the major remaining barriers to realizing the medical benefits of stem cell research might be the risk/benefit balance to patients, the lack of skilled scientists in the field, the source of funding, pressure on researchers to develop commercialized products and to build links with industry, and policies for sharing materials and data and for commercialization in the presence of informed consent.

In conclusion the excitement over the scientific, medical, and financial possibilities of translational stem cell research has been accompanied by ethical concerns therefore there is a need of developing international approaches to address these concerns across the continuum of such research, from bench to bedside and to publication with the attention to global equity and benefit sharing.

Poster Board Number:264**ADVANCEMENT IN REGENERATIVE MEDICINE RESEARCH AND DEVELOPMENT IN JAPAN: NEW FRAMEWORK OF GOVERNMENTAL FUNDED PROJECTS**

Fukushi, Tamami, Okura, Hanayuki, Matsuyama, Akifumi
Platform for Realization of Regenerative Medicine, FBRI, Kobe, Japan

Since the success of human iPSC cell (iPSC) generation in 2007, a variety of research and development (R&D) projects have been promoted to realize regenerative medicine by utilizing iPSCs and other different stem cells in Japan. After Dr. Shinya Yamanaka was awarded the Nobel Prize in Physiology or Medicine in October 2012 for his study on the cell reprogramming mechanism, the reform of the relevant Japanese laws and regulations started to proceed in addition to the reinforcement of R&D investments on the basis of a revised "iPSC Research Roadmap", which was originally published by The Ministry of Education, Culture, Sports, Science and Technology Japan (MEXT) in 2009. While the 2009 edition listed iPSC Research Roadmap of the goals and objectives and the target number of years by tissue/cell to be studied, the revised edition specified the target number of years for each specific item to be performed. This indicates that, due to the advance in the relevant R&D, the whole research has evolved to the stage where the numerical goals can be set in accordance with each tissue/cell research progress for technical or scientific items classified more closely. Similarly, the 11 tissues/cells in the 2009 edition were modified to be more specific and divided in detail in the new one. The revision of "iPSC Research Roadmap" might be linked with governmental investments, in anticipation of a practical use (productization and prevalence in clinical practice) of technological development in regenerative medicine with more rapidity. Indeed, MEXT allocated a total of 9 billion yen (approximately US\$ 90 million) in the fiscal year 2013 budget for operations related to regenerative medicine using stem cells, such as the "Program of the Research Center Network for Realization of Regenerative Medicine". The Prime Minister of the time expressed the intention of long-term support for regenerative medicine research, especially for using iPSCs over the next 10 years. In November 2012, the Ministry of Health, Labour and Welfare Japan (MHLW) selected 4 universities and 2 public institutions to implement the clinical research safety infrastructure development support operation for studies including iPSCs. It was also reported that the total amount of private donations sent to the iPSC Research Fund operated by the Center for iPSC Cell Research and Application (CiRA) at Kyoto University was significantly increased after Yamanaka was awarded the Nobel Prize. Finally the MHLW launched deliberative councils for discussions on the reform and/or introduction of new laws and regulations for swift realization of regenerative medicine utilizing iPSCs.

The current presentation will introduce the detail of trends in Japan on R&D of regenerative medicine between 2012 and 2013 from the viewpoints of the revision of the iPSC Research Roadmap and the top-down approach of R&D investments by the ministries concerned.

Poster Board Number:265**A HISTORY OF THE REGULATORY FRAMEWORK FOR CLINICAL TRANSLATION OF REGENERATIVE MEDICINE IN JAPAN**

Senoo, Hachiro, Ueda, Toshio, Okura, Hanayuki, **Matsuyama, Akifumi**
Platform for Realization for Regenerative Medicine, Foundation for Biomedical Research and Innovation, Kobe, Japan

A medical system is anticipated, where high-quality medical services are accessible without anxieties whenever we are ill. The innovative regenerative medical products, or tissue-engineered medical products, have enabled us to overcome some life-threatening diseases, however, challenges still remain for others. The Ministry of Health, Labour and Welfare, Japan has implemented "The guideline for clinical research using human stem cells" (Notification No.425. 2006. MHLW, Japan.) and has launched on Sep 1, 2006. The major goal of the guideline

is to reinforce and ensure safe on investigational new regenerative medical products. After the guideline had launched till 2013, Minister of MHLW, Japan has approved over 73 protocols in which human somatic stem/progenitor or precursor cells have been applied for patients. In major parts of the approved protocols, bone marrow-derived mesenchymal stem cells have been used for clinical investigation. On 3 Dec 2008, ISSCR has released Guidelines for the Clinical Translation of Stem Cells. The guideline has urged the Japanese government to revise the guideline (Notification No.425. 2006. MHLW, Japan.). The government had invited the academic experts in the field of regenerative medicine, and the committee proposed that the clinical studies using induced Pluripotent Stem cells (iPSCs) shall be allowed, because recent advance of stem cell research hinted us to the epoch in which iPSCs could be used for the clinical studies. On Nov 1, 2010, "The guideline for clinical research using human stem cells" has been revised as Notification No.380. 2010. MHLW, Japan. On the revised guideline, clinical investigators can apply autologous iPSCs as investigational cellular products, and the first clinical trial protocol for the patients with age-related macular degeneration using autologous iPSCs-derived retinal pigmented epitheliums has conditionally approved by MHLW, Japan on July 2013. From Sep 2006, FDA has released a line of guidelines for cellular therapy, and approved embryonic stem cells (ESCs) as INDs for the patients with subacute severe spine injury (Geron) and with age-related macular degeneration (ACT). This news made Japanese government felt the need for revision of "The guideline for clinical research using human stem cells" (Notification No.380. 2010.) and will revise the guideline that clinical investigators be able to apply ESCs- and allogenic iPSCs-derived cellular medicinal products. To accelerate the clinical application of iPSCs-derive cellular medicinal products, the Japanese government decided to change regulatory framework and proposed a bill of Pharmaceutical Affairs Law. In this study, an overview of the history and framework changes will be shown for clinical and/or translational research using human stem cells in Japan.

Poster Board Number:266**THE CURRENT REGULATORY STATUS OF STEM CELL THERAPY AND CLINICAL RESEARCH IN JAPAN**

Kusunose, Mayumi, Takashima, Kayo, Muto, Kaori
Department of Public Policy, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Since Professor Shinya Yamanaka won the Nobel Prize in Physiology or Medicine in 2012, the Japanese Government has been accelerating efforts to develop new legal frameworks relevant to regenerative medicine, especially the field of induced pluripotent stem (iPS) cells. Prime Minister of Japan and His Cabinet have placed stem cell research and regenerative medicine in one of the key elements of Healthcare and Medical Strategy in 2013. Additionally, the government has decided to provide 110 billion yen to support stem cell research over the next 10 years.

According to B.B. Longest, Jr., the public policy making process is a sequence of interactive stages comprising policy formation, implementation, and modifications. It is obvious that purely basic biological findings became the main driving force for the Japan's revitalizing economy strategy.

The purposes of this presentation are to introduce such drastic policy change by the Japanese government and to analyze the current development of the Japanese legal framework regarding stem cell research and clinical practices by utilizing a model of the public policy making process advocated by Longest, and discuss challenges and future agendas in Japan.

According to a report issued by the METI (2013)**, in December 2012, Japan marketed only 2 tissue-engineered medical products, 4 products were under testing, and 66 clinical researches were underway. One of the problems here is that the transition from basic research to drug discovery and marketing does not occur smoothly. Basic research is regulated by the guidelines issued by MEXT. Clinical trials

for application for product approval, called Chiken, are governed by the Pharmaceutical Affairs Law (PAL) and its ministerial ordinances. All other clinical trials (and research using human materials) are regulated by ethical guidelines issued by ministries. These guidelines do not connect research from one stage to the next, which sometimes prevents the smooth and efficient transition of research. In addition, these guidelines sometimes changed in order to keep up with scientific and technological advances and to protect research participants. This makes it difficult for researchers to keep up with the changes. Another probable reason is that the existing PAL does not accurately delineate the characteristics of products derived from human stem cells, especially iPS cells. The application of the same criteria that are used to assess chemically manufactured conventional drugs and other biologics is not enough to ensure the safety and efficacy of iPS products. In addition, this process delays patients' access to the benefits obtained from such research.

Lastly, the Japanese government has recognized the urgent necessity for dealing with unproven stem cell therapy and stem cell tourism. Physicians are allowed to prescribe medical services that are not covered under health insurance, such as front-line therapeutic techniques or cosmetic medicine. These prescriptions are not authorized by MHLW under the existing Medical Practitioners Act and Medical Service Act. In addition, these acts cannot regulate unproven stem cell therapy that is performed in clinical practice under the guise of the physician's discretionary power.

To ameliorate this situation, Japan is in the process of reconstructing regulations and systems for issues ranging from basic research, to clinical practice, and commercial viability relevant to stem cells, including bringing new laws.

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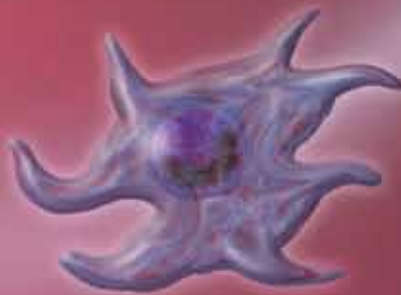
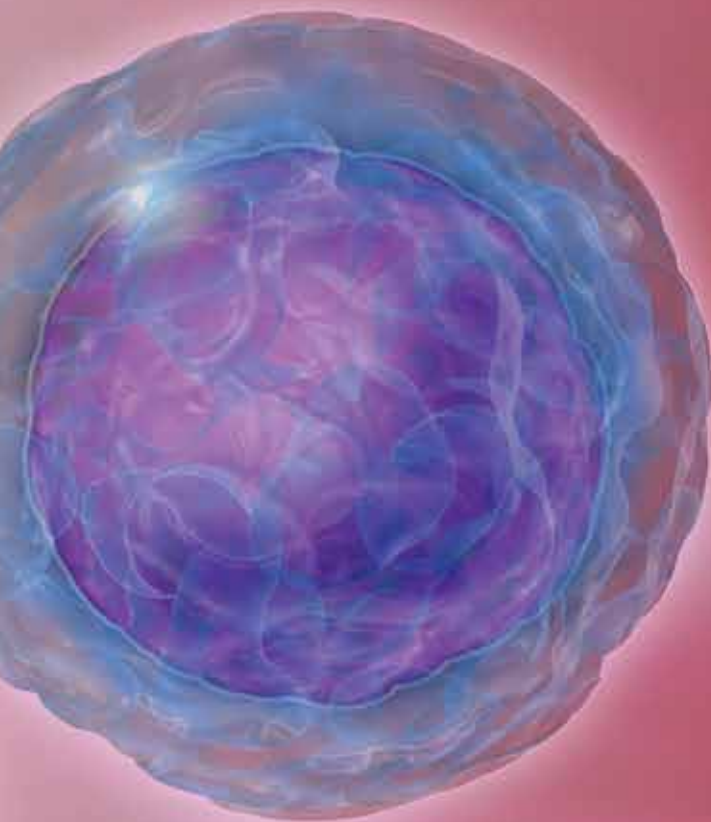
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BD Biosciences
Europe
bdbiosciences.com/eu
Tel.: (+32) 2 400 98 95
Fax: (+32) 2 401 70 94
help.biosciences@europe.bd.com